NASA CELL SCIENCE CONFERENCE 2003

February 20 - 22, 2003
Warwick Hotel
Houston, TX

2003 NASA CELL SCIENCE CONFERENCE

- Annual Investigators Working Group Meeting -

February 20 - 22, 2003

The Warwick Hotel
5701 Main Street, Houston, Texas 77005
Tel: (713) 526-1991 Fax: (713) 526-0359

Sponsored by the Cellular Biotechnology Program, NASA Johnson Space Center (JSC), and the Fundamental Biology Program, NASA Ames Research Center (ARC), in cooperation with the Physical Sciences Division and the Fundamental Space Biology Division of the Office of Biological and Physical Research, NASA Headquarters.

Final Program – Overview

	February 20 Thursday	**February 21** Friday	**February 22** Saturday
Registration / Breakfast	7:30 – 8:30 am	7:00 – 8:00 am	7:00 – 8:00 am
Scientific Sessions	Welcome & Introductions 8:30 – 9:00 am		Session 6: Neoplastic Disease 8:00 – 9:15 pm
	Session 1: Gravity & Mechanical Sensing 9:00 – 11:30 am	Session 4: Cell Culture Technology 8:00 – 11:15 am	Session 7: Immunology 9:45 – 11:15 am
	(Lunch: 11:30-1:00 pm)	(Lunch: 11:15-12:45 pm)	(Lunch: 11:15-12:45 pm)
	Session 2: Motility & Cytoskeleton 1:00 – 2:15 pm	Session 5: Proliferation, Differentiation, and Signal Transduction 12:45 – 3:15 pm	Session 8: Microorganisms 12:45 – 1:45 pm
	Session 3: Sensors & Analytical Equipment 2:30 – 4:00 pm		Session 9: Tissue Engineering 2:00 – 3:30 pm
Exhibits Presentations and Social	4:00 – 5:00 pm		
Business Meeting		3:45 – 4:45 pm	
Plenary Dinner Lecture	7:00 – 9:00 pm		

Final Program - Details

February 20

7:30-8:30 am **Registration & Breakfast**

8:30-9:00 am **Welcome & Introductions**

Session 1 - Gravity & Mechanical Sensing:
Co-chairs: S. Sukharev & T. Hammond

9:00-9:15 am **MA Bucaro[1], IM Shapiro[1], CS Adams[1], K Mukundkrishnan[2], P Ayyaswamy[2], R Gillespie[1], MV Risbud[1]**
[1]Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA , [2]School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, PA
Modeled Microgravity Increases Sensitivity of Osteoblasts to Apoptogens

9:15-9:30 am **BA Horwitz, JS Hamilton, E Warren, CA Fuller**
University of California - Davis
Effects of Hypergravity on Expression of Genes Involved in Regulation of Feeding

9:30-9:45 am **K Mukundakrishnan[1], PS Ayyaswamy[1], M Bucaro[2], M Risbud[2], IM Shapiro[2] , CS Adams[2]**
[1]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA, [2]Department of Orthopaedic Surgery, Thomas Jefferson University
Modeling of Phosphate Ion Transfer to the Surface of Osteoblasts in a Rotating Wall Vessel

9:45-10:00 am **MV Risbud[1], MA Bucaro[1], CS Adams[1], P Ayyaswamy[2], K Mukundkrishnan[2], IM Shapiro[1]**
[1]Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia PA , [2]School of Engineering and Applied Sciences University of Pennsylvania, Philadelphia PA
A Novel Three Dimensional Microcarrier System for Modeling the Effects of Microgravity on Bone Cells

10:00-10:15 am **TG Hammond[1,2], LA Cubano[1], PL Allen[1,2]**
[1]Tulane University Health Sciences Center, [2] VA Medical Center, New Orleans LA
Vitamin D Receptor Translocation Modulates Specific Gene Expression Pathways During Rotating Wall Vessel Culture of Renal Epithelial Cells

10:15-10:30 am **BREAK**

10:30-10:45 am **J O'Donnell, Z Wang, A Chaudhuri**
University of Alabama
Comparison of Catecholamine Regulation in Drosophila Melanogaster Under Hypergravitational and Starvation Stress Conditions

10:45-11:00 am **S Sukharev[1], A Anishkin[1], V Gendel[1], C-S Chiang[1], HR Guy[2]**
[1]Biology Department, University of Maryland, College Park, MD 20742 , [2] Laboratory of Experimental and Computational Biology, DBS, NCI, National Institutes of Health, Bethesda, MD
C-Terminal Domains of the Mechanosensitive Channel MscL form a Cytoplasmic Bundle, but the Assembly is Energetically Uncoupled from the Gate

11:00-11:15 am **K Xu, L Feldman, KL Davis, AJ Sytkowski**
Beth Israel Deaconess Medical Center, Harvard Medical School
Hydrodynamic Shear Forces and Cell Density Affect the Expression of Cell Surface Erythropoietin Receptors

11:15-11:30 am **C Ontiveros, LR McCabe**
Michigan State University 2201BMPS Bldg., East Lansing, MI
RWV Conditions Suppress Osteoblast Phenotype, RunX2 Levels, and AP-1 Transactivation

11:30-1:00 pm **LUNCH BREAK**

Session 2 - Motility & Cytoskeleton:

Co-chairs: Y-L Wang & A. Sundaresan

1:00-1:15 pm **ND Searby[1], W Kraus[3], I Banergee[1], W Vercoutere[1], C Roden[1], M Parra[1], E Holton[1], RK Globus[1,2], E Almeida[1,2]**
[1]NASA Ames Research Center, [2]University of California San Francisco, [3]NASA Astrobiology Academy
Osteoblast Cell Shape and Cytoskeletal Changes during Hypergravity Loading

1:15-1:30 pm **D Montufar-Solis[1], PJ Duke[1], ME Marsh[2]**
[1]Department of Orthodontics, Dental Branch, The University of Texas Health Science Center at Houston, [2]Department of Basic Sciences, Dental Branch, The University of Texas Health Science Center at Houston
Biomineralization and Motility in the Calcifying Alga Pleurochrysis Carterae

1:30-1:45 pm **Y-L Wang[1], M Dembo[2], K Beningo[1], S Munevar[1]**
[1]University of Massachusetts Medical School, [2]Boston University
Mechanical Interactions during Fibroblast Migration in 2D and 3D Environments

1:45-2:00 pm **A Sundaresan[1], D Risin[2], NR Pellis[2]**
[1]Universities Space Research Association, [2]NASA Johnson Space Center, Houston, TX
The Role of Protein Kinase C in Lymphocyte Locomotion: Rescue or Cause?

2:00-2:15 pm **BB Hashemi[1,2], JE McClure[1,2], DL Pierson[1]**
[1]NASA Johnson Space Center, [2]Baylor College of Medicine
The Actin Cytoskeleton: A Molecular Signature for Gravity Sensitivity of Human Blood Cells

2:15-2:30 pm **BREAK**

Session 3 - Sensors & Analytical Equipment:

Co-Chairs: B. Towe & A. Jeevarajan

2:30-2:45 pm **GS Spaulding**
Spin Diagnostics Inc.
Novel Ultrahigh Throughput Flow Cytometry for Cell and Molecular Analysis in UG

2:45-3:00 pm **F Martel[1], L Sun[1], K Slater[1], A Germatsky[1], JA Kane[3], J Lagaz[1], C Preda[2] , G Vunjak-Novakovic[2], J Parrish[1]**
[1]Payload Systems Inc., Cambridge MA, [2]Massachusetts Institute of Technology, Cambridge MA, [3] Polestar Technologies
Optical Oxygen Sensor for use in Perfusion-Based Systems: Long Term Data

3:00-3:15 pm **B Towe[1], C Cooney[1], M Paccini[1], R Martineau[1], P Daydif[2], V Stout[2]**
Arizona State University, Tempe, AZ. [1]Bioengineering Department, and [2] Microbiology Department
Advances in Microculturing of Cells for Space Applications

3:15-3:30 pm **Y Xu[1], J Sun[1], F Gao[1], AS Jeevarajan[1], M Anderson[2]**
[1]Wyle Life Sciences, Houston, TX [2]Biological Systems Office, NASA, JSC, Houston, TX
Continuous Monitoring and Controlling of Glucose Present in Perfused GTSF-2 Medium in a Rotating Wall Perfused Bioreactor Using an On-Line Glucose Sensor

3:30-3:45 pm **H Szmacinski, VJ Pugh**
Microcosm, Inc.
Optical Micro-Sensors for Cellular Studies of Oxygen and Nutrients

3:45-4:00 pm **J Hines[1], B Yost[2], J Fishman[3]**
[1]NASA Ames Research Center, [2]DeFouw Engineering, Mt View, CA., [3]Lockheed-Martin Engineering Services, Moffett Field, CA
Advanced Biological Technologies for Fundamental Space Biology Research

4:00-4:30 pm **SNACK BREAK**

Exhibits Presentations / Social: (4:00 – 5:00)

Presentations by Exhibitors will last 3 – 4 minutes each, starting at 4:00 pm

Plenary Lecture/Dinner:

7:00-9:00 pm Cell Biology in Space: From Basic Science to Biotechnology
Guest Speaker: Dr. Augusto Cogoli

February 21

7:00-8:00 am **Registration & Breakfast**

Session 4 - Cell Culture Technology:

Co-Chairs: S. Gonda & N. Searby

8:00-8:15 am **PI Lelkes[1], N Akhtar[2], E Lelkes[2], L Maltz[3], BR Unsworth[4]**
[1]Drexel University, Philadelphia, PA, [2]University of Wisconsin, Madison, WI, [3]Tel Aviv University, Tel Aviv, Israel, [4]Marquette University, Milwaukee, WI
Generation of Functional Neuroendocrine Tissue Constructs in Rotating Wall Vessel Bioreactors

8:15-8:30 am **S Ohi[1], W Fitzgerald[2], D Riley[3], S Gonda[4]**
[1]Howard University and Hospital, Washington, DC, [2]NASA/NIH Center for Three-Dimensional Tissue Culture, Wyle Laboratories, Bethesda, MD, [3]Medical Col. of Wisconsin, Milwaukee, WI, [4]NASA JSC, Houston, TX
Growth of Hematopoietic Stem Cells in NASA-RWVs: Towards Hematopoietic Stem Cell Therapy for Exploration of Space

8:30-8:45 am **D Montufar-Solis[1], J Hecht [2], J Duke[1]**
[1]Dept of Orthodontics, Univ. TX Dental Branch, UTHSC-Houston, Houston, TX, [2]Dept of Pediatrics, University of Texas Medical School, UTHSC-Houston, Houston, TX
Differentiation of Human Endochondral Cartilage Cultured in the Hydrodynamic Focusing Bioreactor

8:45-9:00 am **EM Troop, PR Bidez, PI Lelkes**
School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104
Culturing Diverse Human and Rodent Cells in the Hydrodynamically Focusing Bioreactor

9:00-9:15 am **JV Valluri, ML Bartholomew, S Hoppe**
Cell Biology Laboratory, Dept. of Biological Sciences, Marshall University, Huntington, WV
Culturing Plant Cells in Hydrodynamic Focusing Bioreactor (HFB)

9:15-9:30 am **F Donovan[1], J Jagger[1], L Sun[5], H Park[2], I Berzin[2], G Vunjak-Novakovic[2], S Walker[3], E Nelson[4], J Kizito[4], C Krebs[5], K Slater[5], D Vandendriesche[3], P Larenas[1], C Havens[1], N Searby[3]**
[1]Lockheed Martin, Moffett Field, CA, [2]Massachusetts Institute of Technology, Cambridge MA, [3]NASA Ames Research Center, Moffett Field CA, [4]NASA Glenn Research Center, Cleveland, OH, [5]Payload Systems Inc, Cambridge, MA
Cells Go with the Flow: Characterizing the Flow and Choosing Perfusion and Mixing Rates for Variable Gravity Experiments

9:30-9:45 am **BREAK**

9:45-10:00 am **JM Ramsey[1], CT Culbertson[2], MA McClain[1], Luke Tolley[1], SR Gonda[3], SC Jacobson[1]**
[1]Oak Ridge National Laboratory, [2]Kansas State University, [3]NASA Johnson Space Center Cellular Biotechnology Program
Microfluidic Devices for the Chemical Analysis of Single Cells

10:00-10:15 am **JC Vellinger, E Taylor, NS Logan, MA Kurk, A Jones, D Kennedy, P Todd**
SHOT, 7200 Highway 150, Greenville, IN 47124
Cassette-Based Cell Culture Research Tools For Microgravity Experiments

10:15-10:30 am **K Mukundakrishnan[1] , PS Ayyaswamy[1] , M Bucaro[2] , M Risbud[2] , IM Shapiro[2], CS Adams[2]**
[1]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA, [2]Department of Orthopaedic Surgery, Thomas Jefferson University.
Motion of a Porous Spherical Microcarrier in a Rotating Wall Vessel

10:30-10:45 am **DH Slentz[1], GA Truskey[2], WE Kraus[1,2]**
[1]Department of Medicine, Duke University Medical Center, [2]Department of Biomedical Engineering, Duke University
Three Dimensional Muscle Cultures as a Model for Ground Based Research using the Synthecon Rotating Cell Culture System (RCCS)

10:45-11:00 am **S Geffert, S Kleis**
University of Houston
Fluent Modeling for the HFB-S

11:00-11:15 am **S Kleis, S Geffert**
University of Houston
Fluid Dynamic Investigations of the HFB-S

11:15-12:45 pm **LUNCH BREAK**

Session 5 - Proliferation, Differentiation, and Signal Transduction:

Co-Chairs: N. Pellis & M. Hughes-Fulford

12:45-1:00 pm **KS Cole[1], A Sundaresan[2], NR Pellis[3], SM Green[1]**
[1]University of Louisiana at Lafayette, [2]Universities Space Research Association, [3]NASA-Johnson Space Center
Abnormal Developmental Effects Following Prolonged Embryonic Exposure to a Rotating Cell Culture System (RCCS) Environment, in the Cypriniform Fish, Rivulus Marmoratus

1:00-1:15 pm **S Saporta[1, 2, 3], AE Willing[1, 2, 3], PR Sanberg[2, 3], LO Colina[1], J Hushen[1], DF Cameron[1, 3]**
[1]Department of Anatomy, [2]Department of Neurosurgery, [3]Center for Aging and Brain Repair, University of South Florida College of Medicine
Rapid Expression of Neuronal Markers by Sertoli-Neuron-Aggregated Cells (SNAC) Tissue Constructs

1:15-1:30 pm **LJ Kundakovic[1], S Pretorius[2],G Sun[3], P Larenas[3], J Rask[3], N Searby[4], J De Luis[2], D Vandendriesche[4] , G Vunjak-Novakovic[1]**
[1]Massachusetts Institute of Technology, Cambridge MA, [2]Payload Systems Inc., Cambridge MA, [3] Lockheed Martin, Moffet Field, CA, [4] NASA Ames Research Center, Moffett Field CA
Myoblast Differentiation Under Flow Conditions

1:30-1:45 pm **W Vercoutere[1], C Roden[1], M Parra[1], E Holton[1], ND Searby[1], RK Globus[1,2], EA Almeida[1,2]**
[1]NASA Ames Research Center , [2]University of California San Francisco
Integrin-Mediated Bone Cell Proliferative Response to Constant Hypergravity

1:45-2:00 pm **EAC Almeida[1,2], D Amblard[1,2], MCH van der Meulen[3], CD Damsky[2], JB Kim[2], Y Nishimura[1,2], UT Iwaniec[4], TJ Wronski[4], RK Globus[1,2]**
[1]NASA Ames Research Center, [2]University of California, San Francisco, [3] Cornell University, [4] University of Florida, Gainesville
Transgenic Mouse Model of Altered Integrin Signaling in Osteoblasts: Skeletal Phenotype and Responses to Disuse

2:00-2:15 pm **RK Singh[1], X Chen[1], D Hamrick[1], GP Siegal[2]**
[1]Diversified Scientific Inc., Birmingham, AL, [2]Department of Pathology, University of Alabama at Birmingham AL
Endothelial Cell Activation and Differentiation in Defined 3D Human Biomatrix Culture Models

2:15-2:30 pm **SC Stout, GB Clark, SJ Roux**
Molecular Cell and Developmental Biology, The University of Texas at Austin, Austin TX
The use of RNAI to Investigate Gene Function During Polarity Development in Ceratopteris RichardII

2:30-2:45 pm **M Hughes-Fulford[1], W Sugano[1], G Cogoli[2]**
[1]Lab of Cell Growth, UCSF, NCIRE and VAMC, San Francisco, CA, [2]Space Biology, ETH Zurich, Switzerland
Early T-Cell Activation Mediated by PKA/MAPK

2:45-3:00 pm **CE Helmstetter, V LeBleu, M Thornton, A Romero**
Department of Biological Sciences, Florida Institute of Technology, Melbourne FL.
Cell Cycle Properties Of Mouse L1210 Cells In A Rotating Culture Vessel

3:00-3:15 pm **W Ma[1], W Fitzgerald[2,3], S Chen[2,3], TJ O'Shaughnessy[1], D Maric[4] , JL Barker[4] ,HJ Lin[1]**
[1]Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, D.C. 20375, [2]Wyle Laboratories, Houston TX, [3]NASA/NIH Center for Three Dimensional Tissue Culture, NICHD, [4]Lab of Neurophysiology, NINDS, National Institutes of Health, Bethesda, MD 20892
Modeled Microgravity Promotes Aggregation and Proliferation of Neural Precursor Cells Cultured in Three-Dimensional Collagen Gels

3:15-3:45 pm **SNACK BREAK**

Business Meeting:

3:45-4:45 pm State of the Program

Neal Pellis, Biological Systems Office Chief, Biotechnology Program – NASA/JSC
Ken Souza, Senior Staff Scientist, Fundamental Space Biology Program – NASA/ARC

February 22

7:00-8:00 am **Registration / Breakfast**

Session 6 - Neoplastic Disease:

Co-Chairs: V. Chopra & J. Jessup

8:00-8:15 am **V Chopra[1], TV Dinh[1], TG Wood[1], NR Pellis[2], EV Hannigan[1]**
[1]Division of Gynecologic-Oncology, Department of Obstetrics and Gynecology, Molecular Science Center, UTMB, Galveston, Texas 77555-0587, [2] Cellular Biotechnology, JSC-NASA, Houston, TX
Characteristics Of Tumor and Host Cells in 3-D Analog Microgravity Environment

8:15-8:30 am **K O'Connor, H Song, C Giordano, S Clejan, O David**
Tulane University and Medical School
Spatial Organization of Prostate Cancer Spheroids in a Rotating-Wall Vessel and Liquid-Overlay Culture

8:30-8:45 am **JL Becker[1], RH Widen[2]**
[1]Department of Obstetrics and Gynecology, University of South Florida, [2]Tampa General Hospital, Clinical Patholology Laboratory, Tampa, FL
Drug Responsiveness of Human Ovarian Tumor Cells Cultured as Three- Dimensional Aggregates

8:45-9:00 am **JM Jessup[1], NR Pellis[2]**
[1]Georgetown University Medical Center, Washington, DC, [2]NASA/JSC, Houston, TX
ISS Cell Science Research: Lessons Learned from CBOSS

9:00-9:15 am **AD Kulkarni[1], M Taga[1], K Yamauchi[1], L Furian[1], J Odle[1,3], A Sundaresan[2,3], NR Pellis[3], RJ Andrassy[1]**
[1]The UTHSC-H, Surgery Dept., Houston, TX 77030, [2]USRA, Houston, TX, [3]JSC, NASA
Tumor Cellular Response and Growth in Ground-Based Microgravity Analogs and Role of Nutrition in Microgravity

9:15-9:30 am **S Sung, J Shu, F Jin, SD Lim, P Nelson, M Amin, FF Marshall, LWK Chung**
Emory University School of Medicine, Atlanta, Georgia 30322
Identification of Genes Activated in Tumor-Associated Stroma: Three-Dimensional Co-Culture Cell Models Recapitulated Gene Expression Profiles in Clinical Prostate Cancer

9:30-9:45 am **BREAK**

Session 7 - Immunology:

Co-Chairs: B. McIntyre & D. Risin

9:45-10:00 am **BF Andruss[1], SA Lopez[1], M Arya[2], B Anvari[2], BW McIntyre[1]**
[1]Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX, [2]Department of Bioengineering, Rice University, Houston, TX
Force Measurements with Molecular Tweezers Reveal Reduced Integrin-Mediated Adhesion in Human Lymphocytes Cultured in Simulated Microgravity

10:00-10:15 am **L Rutzky[1], S Bilinski[2], M Kloc[1], H Zhang[1], T Phan[1], S Katz[1], S Stepkowski[1]**
[1]Department of Surgery, University of Texas Medical School, Houston, TX, [2]Institute of Zoology, Jagiellonian University, Crakow, Poland
Effect of Modeled Microgravity on Rat Pancreatic Islets for Xenotransplantation

10:15-10:30 am **JHD Wu, CY Chen, M Heckman, YG Chen, A Mantalaris**
Department of Chemical Engineering, University of Rochester, Rochester, NY
Circadian Rhythm of Hematopoiesis

10:30-10:45 am **J Zimmerberg, W Fitzgerald, J Grivel, S Chen, L Margolis**
Immune System Function and Dysfunction in the Rotating Wall Vessel and in Microgravity

10:45-11:00 am **PS Blank[1], L Sappelsa[1], J Farrington[1], P Bungay[2], and J Zimmerberg[1]**
[1]NASA-NIH Center for Three Dimensional Tissue Culture, Laboratory of Cellular and Molecular Biophysics, NICHD, [2]Division of Bioengineering and Physical Sciences, ORS, NIH
Evaluation of Ground-Based Mixing in Teflon Culture Bags: Implications For μ-gravity Research

11:00-11:15 am **PH Duray, S Yin, Y Ito, W Fitzgerald, L Bezrukov, J Zimmerberg, L Margolis**
Rotating Wall Vessel as a Model for Infectious Disease

11:15-12:45 pm **LUNCH BREAK**

Session 8 - Microorganisms:

Co-Chairs: C.A. Nickerson & J. DiRuggeiro

12:45-1:00 pm **L Sun[1], S Pretorious[1], J Lagaz[1], C Preda[4], F Donovan[2], N Searby[3], C Havens[2], D Vandendriesche[3], J de Luis[1], J Parish[1], G Vunjak-Novakovik[4]**
[1]Payload Systems Inc, Cambridge, MA, [2]Lockheed Martin, Moffett Field, CA, [3] NASA ARC, CA, [4]MIT, Cambridge, MA
Ground Based Studies of Saccharomyces Cerevisiae Yeast Growth in the Cell Culture Unit

1:00-1:15 pm **JW Wilson[1], R Ramamurthy[1], S Porwollik[2], M McClelland[2], T Hammond[1], P Allen[1], CM Ott[3], DL Pierson[4], CA Nickerson[1]**
[1]Tulane University School of Medicine, [2]Sidney Kimmel Cancer Center, [3]EASI/Wyle Laboratories, [4]NASA-Johnson Space Center
Microarray Analysis Identifies Salmonella Genes Belonging to the Low-Shear Modeled Microgravity Regulon

1:15-1:30 pm **C Iloanusi, K Kunst, S Borjk, J DiRuggiero**
Dept of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland
Molecular Basis For Survival of the Halophile Halobacterium NRC1 in Space Conditions

1:30-1:45 pm **E dL Pulcini, SC Broadaway, BH Pyle**
Montana State University Bozeman
STS-107 Experiment BACTER: Ground Experiments

1:45-2:00 pm **BREAK**

Session 9 - Tissue Engineering:

Co-Chairs: A. Fertala & A. Rajan

2:00-2:15 pm **L.E. Freed[1], J. Seidel[1], G. Altman[2], J. Boublik[1], D. Kaplan[2], G. Vunjak-Novakovic[1]**
[1]Division of Health Sciences and Technology, M.I.T. Cambridge, MA, [2]Tufts University; Medford, MA
Functional Tissue Engineering: Roles of Biophysical Factors

2:15-2:30 pm **G Vunjak-Novakovic, M Radisic, H Park, J Boublik, LE Freed**
Massachusetts Institute of Technology
Biomimetic Approach to the Tissue Engineering of Functional Myocardium

2:30-2:45 pm **I Majsterek, E McAdams, A Fertala**
Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA
Characterization of Novel Recombinant Collagen-Like Proteins

2:45-3:00 pm **AS Rajan[1], A Patel[1], S Navran[2]**
[1]Baylor College of Medicine, [2]Synthecon Inc.
Development of a Modified Perfused Bioreactor for Analysis of Hormone Secretion in Islet Cell Cultures

3:00-3:15 pm **V.I. Khaoustov[1], D.Risin[2], N.R.Pellis[2], B.Yoffe[1]**
[1]Baylor College of Medicine, Houston, TX 77030, [2]NASA, Biotechnology Program, Houston, TX 77058
Liver Tissue Engineering in Microgravity Environment

3:15-3:30 am **PJ Lupo[1], K Emami[2], SR Gonda[1]**
[1]NASA/Johnson Space Center, Houston, TX, [2]Wyle Laboratories, Houston, TX
Gene Expression Profiling of Human Renal Cortical Epithelial Cells at Three Stages of Tissue Engineering in the Hydrodynamic Focusing Bioreactor (HFB)

2003 NASA CELL SCIENCE CONFERENCE

PROGRAM ABSTRACTS

-Annual Investigators Working Group Meeting –

February 20-22, 2003
Park Plaza Warwick Hotel
Houston, Texas

Sponsored by the Cellular Biotechnology Program, NASA Johnson Space Center (JSC), and the Fundamental Biology Program, NASA Ames Research Center (ARC), in cooperation with the Physical Sciences Division and the Fundamental Space Biology Division of the Office of Biological and Physical Research, NASA Headquarters.

MODELED MICROGRAVITY INCREASES SENSITIVITY OF OSTEOBLASTS TO APOPTOGENS

M. A. Bucaro[1], I. M. Shapiro[1], C. S. Adams[1], K. Mukundkrishnan[2], P. Ayyaswamy[2], R. Gillespie[1], M.V. Risbud[1]
[1]Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA , [2]School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, PA

Microgravity experienced by astronauts during extended space flight is linked to bone loss. It is hypothesized that microgravity induces bone cell apoptosis and results in a generalized osteopenia. Using NASA-approved high aspect-ratio rotating vessels (HARV's) and a novel three-dimensional microcarrier system we have examined the relationship between microgravity and apoptosis in osteoblasts. MC3T3 cells were encapsulated in alginate beads and subjected to modeled microgravity for 5 days at a low rotational speed (8 rpm). Osteoblasts in alginate microcarriers, cultured in a horizontal HARV (vertical axis of rotation), were used as a control. Cells were monitored for their viability using Mito-tracker red (membrane potential) and Cell-tracker green (thiol status) dual labeling. At the end of 5 days, cells were challenged for 3 hrs in serum-free medium with the apoptogen, staurosporin (1 uM). Cells were harvested by dissolving the alginate beads and collected by centrifugation. To identify apoptotic osteoblasts, flow cytometry was performed using cells dual labeled with Annexin-V and propidium iodide. Cells were also studied for expression of bone-specific integrins by flow cytometry. Total RNA and protein was isolated to evaluate expression of genes known to modulate apoptosis. The fluorescent probes indicated that the cells were healthy. However, it was noted that microgravity consistently down regulated expression of beta-1, beta-3, alpha-v, and alpha-5 integrin subunits. When challenged with the apoptogen, cells exhibited accelerated apoptosis. RT-PCR and Western blot analyses indicated modulation of both pro-and anti-apoptotic molecules. The results of the investigation clearly indicate that modeled microgravity sensitizes osteoblasts to apoptogens. This finding lends strong support for the hypothesis that microgravity induces bone cell apoptosis.

(Supported by NASA Grant NRA 00-HEDS)

EFFECTS OF HYPERGRAVITY ON EXPRESSION OF GENES INVOLVED IN REGULATION OF FEEDING

B. A. Horwitz, J. S. Hamilton, E. Warren, C.A. Fuller
University of California - Davis

Exposure to altered gravitoinertial fields result in alterations in the regulation of energy balance. We are examining the cellular basis for these alterations by evaluating the relationship between adiposity, leptin signaling, and energy intake. Specifically, we are testing the hypothesis that 2G exposure alters expression of hypothalamic genes whose products modulate food intake and thus energy balance. Using real-time (quantitative) PCR techniques, we are measuring mRNA levels of preproneuropeptide Y (ppNPY), agouti related peptide (AGRP), cocaine-and-amphetamine-regulated transcript (CART), and proopiomelanocortin (POMC) in the arcuate nucleus; levels of orexin and melanin concentrating hormone (MCH) mRNA in the lateral hypothalamus; and levels of NPY-Y1 and NPY-Y5 receptor mRNA in the paraventricular nucleus. The experiment involves 3 groups of adult male rats (22-24 weeks of age): 2G exposed, fed ad libitum; 1G exposed, fed ad libitum; and 1G exposed, fed the same amount of food as the 2G rats. Animals were sacrificed at weeks 1, 2, and 8 of exposure; their brains were removed; and hypothalamic regions to be examined were microdissected. The analyses of mRNA levels, adiposity, and circulating leptin is underway.

(Supported by NASA Grant NAG2-1494)

MODELING OF PHOSPHATE ION TRANSFER TO THE SURFACE OF OSTEOBLASTS IN A ROTATING WALL VESSEL

K. Mukundakrishnan[1], P. S. Ayyaswamy[1], M. Bucaro[2], M. Risbud[2], I. M. Shapiro[2], C. S. Adams[2]
[1]Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA 19104-6315, [2]Department of Orthopaedic Surgery, Thomas Jefferson University

We have numerically modeled the mass transfer associated with a NaPi transporter in regard to an osteoblast culture carried out in a rotating wall bio-reactor (RWV – High aspect ratio vessel HARV). In the modeling, our goal has been to ascertain the extent of phosphate ion (Pi) accumulation at the surface of the osteoblast. Since the osteoblasts in our system are cultured on spherical microcarriers, the Pi accumulation that we predict from the model would correspond to that accumulating at the surface of the microcarrier. In order to predict the Pi accumulation, conservation equation (convection-diffusion)for mass transfer was solved in conjunction with the equation for microcarrier motion. The mass transfer equation is governed by reaction-kinetics boundary condition (Michaelis-Menten Kinetics).

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A NOVEL THREE DIMENSIONAL MICROCARRIER SYSTEM FOR MODELING THE EFFECTS OF MICROGRAVITY ON BONE CELLS

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Studies of bone cell function in modeled microgravity employ microcarriers suspended within a rotating bioreactor with cells attached to their outer surface. A disadvantage of this system is that a limited number of microcarriers can be used; this limits the number of cells that can be studied. Furthermore, microcarriers often collide with other particles and with the walls of the culture vessel. Importantly, cells on the the microcarrier surface form a monolayer that fails to model the three-dimensional characteristics of bone. To overcome the disadvantages inherent in the use of conventional microcarriers, we have designed a new carrier in which cells are immobilized in a three dimensional polymer network. Human primary bone cells and MC3T3-E1 cells were suspended in an alginate-fibronectin solution at a concentration of 2-5 million cells per ml and sprayed by a droplet generator into a cross-linking solution (105 mM $CaCl_2$) to generate spherical beads with cells immobilized in a three-dimensional network. By adjusting the airflow and the polymer injection rate, the microcarrier diameter was varied from 100 to 500 microns. Cells in the microcarriers were cultured in a rotating wall NASA-approved vessel at 8 rpm for 5-14 days and evaluated by confocal microscopy using Mitotracker red and Celltracker green dual labeling. It was found that the cells remained viable and maintained their thiol redox status throughout the culture period. Surprisingly, when challenged, these cells were found to display increased sensitivity to apoptotic agents. Numerical modeling indicated that the bone cells experience a very low shear environment with a small net acceleration vector. It was concluded that the alginate microcarriers provide an optimum system to study the effects of microgravity on bone cells. Moreover, this approach could easily be adapted for use with many other cell types.

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VITAMIN D RECEPTOR TRANSLOCATION MODULATES SPECIFIC GENE EXPRESSION PATHWAYS DURING ROTATING WALL VESSEL CULTURE OF RENAL EPITHELIAL CELLS

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Rotating wall vessel culture of human renal epithelial cells is accompanied by time dependent changes in multiple gene expression pathways. To define the molecular mechanisms underlying these changes, we assayed for transcription factors, which translocated from the cytosol to the nucleus prior to the gene expression changes. In particular, vitamin D receptor abundance fell 3 fold in the cytosol and increased 3 fold in the nucleus, with peak changes observed one hour after initiation of rotating wall vessel culture. To systematically define which gene expression pathway changes were vitamin D receptor dependent, we first developed tools to stimulate and inhibit vitamin D receptor translocation. Addition of 1-25-diOH Vitamin D3 induced nuclear translocation of the receptor. We identified a peptide representing one of three candidate nuclear translocation motifs in the vitamin D receptor, adjacent to the leucine zipper, which inhibits vitamin D receptor translocation (whether due to vitamin D treatment or rotating wall vessel culture). Equipped with these reagents we cultured renal cells for 2 hours in the rotating wall vessel with vitamin D receptor translocation stimulation and inhibition. We performed gene array analysis after 2 hours of renal cell culture. Of the gene expression pathways which changed with vitamin D receptor modulation some were reciprocal when the receptor was increased or decreased, while other pathways were intervention specific. This data defines specific gene expression pathway changes which vitamin D receptor transcription factor translocation mediates during rotating wall vessel culture of renal cells.

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COMPARISON OF CATECHOLAMINE REGULATION IN DROSOPHILA MELANOGASTER UNDER HYPERGRAVITATIONAL AND STARVATION STRESS CONDITIONS

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Drosophila melanogaster exhibits a response to diverse types of environmental stress that includes an up-regulation of catecholamine production. The mechanisms by which this stress pathway is regulated closely parallels those of mammals, suggesting that *Drosophila* represents an excellent model system for detecting and analyzing responses to environmental change. We are comparing the effects of hypergravity and starvation stress on the activity and regulation of three key genes in catecholamine regulation. We are also examining the consequences of perturbation of catecholamine pools by mutations in these genes on the ability of this organism to withstand these extreme environmental changes. We observe comparable effects of nutritional deprivation and stress on mutant strains. Strains carrying mutations in Catecholamines-up, a negative regulator of catecholamine synthesis, have elevated catecholamine pools under non-stress conditions and enhanced ability, relative to wild type strains, to withstand resistance to both hypergravitational and starvation conditions. Strains carrying mutations in genes required for catecholamine synthesis (encoding key enzymes, GTP cyclohydrolase I and tyrosine hydroxylase) have reduced catecholamine pools under non-stress conditions and diminished capacity to withstand starvation or hypergravity conditions, relative to wild type strains. In all strains, females have a greater capacity to withstand both environmental conditions than do males. While resistance or sensitivity to hypergravity parallels resistance or sensitivity to starvation in this analysis of mutant strains, recent results suggest that the responses to these two conditions are regulated differently. During exposure to hypergravity conditions, the level of Catecholamines- up protein, which is a post-translational regulator of GTP cyclohydrolase and tyrosine hydroxylase, is decreased, while tyrosine hydroxylase protein levels appear unchanged. This response is consistent with post-translational regulation of the pathway under these conditions. In contrast, during starvation, the level of Catecholamines-up protein appears unchanged, while that of tyrosine hydroxylase appears to increase, consistent with a mode of transcriptional regulation of the pathway. Analysis of RNA levels is in progress to test this interpretation of the observations.

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C-TERMINAL DOMAINS OF THE MECHANOSENSITIVE CHANNEL MscL FORM A CYTOPLASMIC BUNDLE, BUT THE ASSEMBLY IS ENERGETICALLY UNCOUPLED FROM THE GATE

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C-terminal (S3) domains are conserved within the MscL family of bacterial mechanosensitive channels, but their function remains unclear. The X-ray structure of MscL from Mycobacterium tuberculosis (TbMscL) revealed cytoplasmic domains forming a pentameric bundle (Chang et al., Science 282:2221). The helices, however, have an unusual orientation in which hydrophobic sidechains face outside while charged residues face inside, apparently due to specific crystallization conditions. Based on the structures of TbMscL and of pentameric cartilage protein COMP, we modeled the resting conformation of the C-terminal region of E. coli MscL to better satisfy the hydrophobicity criteria, with sidechains of conserved aliphatic residues all inside, and multiple polar residues forming salt bridges outside the bundle. A molecular dynamic simulation confirmed the stability of this conformation and predicted distances for disulfide trapping experiments. The single cysteine mutants L121C, I125C, and L128C all formed dimers under ambient conditions and more so in the presence of an oxidant. The double-cysteine mutants L121C/L122C and L128C/L129C often cross-link into a pentameric structure, suggesting close proximity of these residues, consistent with the model. Patch-clamp examination of these double mutants under moderately oxidizing or reducing conditions revealed similar activities indicating that the bundle cross-linking neither prevents the channel from opening nor changes the thermodynamic parameters of gating. Destabilization of the bundle by substituting conservative leucines with small polar residues increased the occupancy of subconducting states. We conclude that S3 domains are stably associated in both closed and open conformations, but their association is energetically uncoupled from the gate. The functional role of the bundle-like assembly is discussed.

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HYDRODYNAMIC SHEAR FORCES AND CELL DENSITY AFFECT THE EXPRESSION OF CELL SURFACE ERYTHROPOIETIN RECEPTORS

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The hydrodynamic shear forces experienced by biologic systems exposed to the microgravity environment of space flight are markedly different from those experienced in the gravitational environment of earth. Humans and other mammals have exhibited anemia and relative immune deficiency after prolonged exposure to microgravity. Microgravity and hydrodynamic shear have been shown to affect several biologic processes, including intracellular signaling transduction pathways and gene expression. However, the effect of shear on cell surface receptor expression and function is not well understood. Previously, we reported that erythroid cell growth and erythropoietin (Epo)-induced differentiation are markedly reduced when cells are grown in the NASA Rotating Wall Vessel (RWV) bioreactor (A.J. Sytkowski, K.L. Davis. In Vitro Cell Dev Biol. Anim. 2001). We now report that both hydrodynamic shear and cell density influence cell surface Epo receptor (EpoR) expression, assessed by binding of 125I-labeled human Epo. When BaF3 cells expressing the human EpoR were cultured at a high cell density, EpoR numbers were significantly lower than when these cells were cultured at a low density ($p<0.05$). A qualitatively similar effect was observed when cells were grown either in tissue culture flasks, with the standard cell culture shear forces, or in the low shear environment of the RWV. Importantly, the magnitude of this effect on EpoR expression was markedly influenced by changes in hydrodynamic shear, I.e., RWV culture. In flasks, receptor numbers on cells grown at low density were approximately 1.5-2 fold higher than on cells grown at higher density. In contrast, when cells were grown in the RWV, EpoR expression was 3-4 fold higher on cells grown at low density and was markedly diminished on cells grown at higher density. These effects of shear and cell density may help explain the differing results of ligand-receptor interaction seen in studies carried out in microgravity and could play a role in the effects of microgravity on hematopoietic cells, resulting in space anemia and relative immune deficiency.

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RWV CONDITIONS SUPPRESS OSTEOBLAST PHENOTYPE, RUNX2 LEVELS, AND AP-1 TRANSACTIVATION

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Conditions of disuse such as bed rest, space flight, and immobilization result in decreased mechanical loading of bone, which is associated with reduced bone mineral density and increased fracture risk. Mechanisms involved in this process are not well understood but involve the suppression of osteoblast function. To elucidate the influence of mechanical unloading on osteoblasts, a rotating wall vessel (RWV) was employed as a ground based model of simulated microgravity. Mouse MC3T3-E1 osteoblasts were grown on microcarrier beads for 14 days and then placed in the RWV for 24 hours. Consistent with decreased bone formation during actual spaceflight conditions, alkaline phosphatase and osteocalcin expression were decreased by 80% and 50%, respectively. In addition, runx2 expression and AP-1 transactivation, key regulators of osteoblast differentiation and bone formation, were reduced by more than 60%. Further analysis of AP-1 member expression demonstrated differences in AP-1 member levels in RWV compared to g controls. These finding suggests that simulated microgravity could promote dedifferentiation and/or transdifferentiation to alternative cell types; however, markers of adipocyte, chondrocyte, and myocyte lineages were not induced by RWV exposure. Taken together our results indicate that simulated microgravity may suppress osteoblast differentiation through decreased runx2 and AP-1 activities. Studies are underway to determine the mechanism for this response.

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OSTEOBLAST CELL SHAPE AND CYTOSKELETAL CHANGES DURING HYPERGRAVITY LOADING

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In previous studies, we have shown that bone forming cells, osteoblasts, respond to centrifugation-induced hypergravity with altered focal adhesions, cell shape, and cytoskeletal network height. However, these changes were observed after the osteoblasts were removed from the centrifuge for fixation. We asked whether these responses would be different if the cells were fixed on-board the centrifuge while still experiencing the hypergravity load, rather than after the centrifuge has stopped and during hypergravity recovery. To answer this question, we developed a pair of automated centrifuge fixation units (ACFU). The ACFUs delivers specified quantities of fixative to up to eight different cell chambers at one time. The fixation delivery rate, schedule, and volume can be pre-programmed prior to experiment initiation. Tests confirmed uniform fixative delivery and adequate fixation. In preliminary experiments, cultured primary fetal rat osteoblasts were grown to near confluence and centrifuged at 10 times gravity (10-g) for 3 hours using the NASA Ames Incu-fuge (cell culture incubator/centrifuge), and1-g controls were placed in an adjacent incubator. Cells were fixed during and immediately after centrifugation. Microtubules and nuclei were fluorescently labeled and analyzed by confocal microscopy, and differences were noted in microtubule morphology and overall microtubule network height in both on-board and recovery cells as compared to the 1-g controls. In other preliminary experiments, rat osteosarcoma cells (ROS 17/2.8) were trypsinized, replated for 30 minutes, and centrifuged at 50-g for 30 minutes using the NASA Ames Incu-fuge with 1-g controls placed in an adjacent incubator. Differences were noted in the actin cytoskeleton as well. In conclusion, the osteoblast cytoskeleton responds dynamically to the changes in loading in a rapid fashion. This model system looking at the application of hypergravity as well as recovery at the cellular level may prove analogous to spaceflight studies performed in vivo in rats studied both on-board the Space Shuttle or International Space Station as well as after landing.

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BIOMINERALIZATION AND MOTILITY IN THE CALCIFYING ALGA PLEUROCHRYSIS CARTERAE

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The coccolithophorid Pleurochrysis carterae provides an excellent model to study the relationship of biomineralization and morphology to motility and gravitaxis. The Wintrack 2000 software was used to analyze cell motility on samples from cultures grown under various conditions, including T-flasks with and without gas exchange, and in stationary vs rotated high aspect ratio vessel (HARV) bioreactors. Cells from these cultures were injected into a Rose chamber for analysis of cell motility, which showed that cells in young growing cultures, with low cell concentration, swam randomly. As cell density increased in the parent cultures, bioconvection was established in the Rose chamber. Distinct large areas of cells swimming up and narrow streams of cells moving down were observed during bioconvection, which was sometimes visible to the naked eye in stationary cultures (bioreactor or T-flasks), but not in the rotating ones. Movement analysis also showed that even after bioconvection currents were established, random movement still existed in less concentrated regions of the Rose chambers. In rotating bioreactor cultures, cell numbers increased exceptionally more than in any other culture systems tested to date. Although no convection currents were visible while these were rotating, within minutes of stopping rotation of 2 week cultures, bioconvection currents appeared. No bioconvection occurred after stopping the 8 week cultures; furthermore, scarcely any cells were swimming even after 5 hrs of observation. Aging of the cultures increased cell adhesiveness, aggregation, and sedimentation, depleting the number of swimmers in the population and resulting in loss of bioconvection. Therefore, in P. carterae, cell concentration seems to be responsible for the oriented movement, due to its effect on the establishment of bioconvection. Coccolith formation in the various environments is being evaluated. Observations indicate that cells cultured for 8 weeks in the bioreactor have fewer coccoliths than either those cultured for 2 weeks in the bioreactor, or those cultured in T-flasks for 8 weeks. Further studies are needed to determine if the difference in number of coccoliths is related to differences in oxygenation, rpms, or culture age.

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MECHANICAL INTERACTIONS DURING FIBROBLAST MIGRATION IN 2D AND 3D ENVIRONMENTS

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Complex mechanical interactions take place during fibroblast migration. Mechanical signals exerted on the cell membrane are able to elicit profound responses in cell morphology, migration and growth. Using 3T3 fibroblasts cultured on flexible, polyacrylamide substrates, we found that extracellular calcium and stretch-activated ion channels play a key role in mediating the response of fibroblasts to mechanical signals. Blocking of stretch-activated channels with gadolinium caused decreases in traction forces and dissociations of vinculin and phosphotyrosine from focal adhesions, without inhibiting the spread morphology or local protrusive activities. Local applications of gadolinium indicated that channels in the frontal region are responsible for the regulation of traction forces, likely in conjunction with the exertion of active traction forces to the substrate. We have recently extended our studies to a simulated 3D environment where cells are cultured within a sandwich of polyacrylamide substrates. Compared to those cultured on 2D polyacrylamide sheets, cells in the 3D environment showed striking differences in morphology and motility. Most cells became elongated, showing either a spindle shape or a branched morphology and no lamellipodium. Forward migration was propelled through the extension of thin processes. By varying the flexibility of the substrates, we found that lack of dorsal adhesion, or adhesions to soft substrates, may send a global stimulatory signal for cell spreading and migration. These properties were observed only when the top substrate was coated with fibronectin, indicating that dorsal physical contact alone was not sufficient and that receptor engagement was essential. Our approach allows visualization of live cells in a physiologically relevant environment, and suggests a dramatic difference in cellular behavior when receptor engagement is no longer limited to the ventral surface. Moreover, calcium entry through stretch-activated channels likely plays an important role in mediating active and passive responses to mechanical signals.

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THE ROLE OF PROTEIN KINASE C IN LYMPHOCYTE LOCOMOTION: RESCUE OR CAUSE?

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Space travel and long-term space residence such as planned in the ISS era may invoke unique burdens on the immune system. An optimal immune response is required to counteract and withstand exposure to pathogens and disease. Important lymphocyte functions such as activation and locomotion are impaired in modeled microgravity as well as in space. Modeled microgravity (MMG) exerts effects on different cells in different ways. These effects could be selective, species and cell type specific. In human lymphocytes, calcium-independent Protein Kinase C (PKC) isoforms are differentially expressed in MMG. PKC-delta and -epsilon were found to be down-regulated by more than 50%, while calcium-dependent PKC-alpha expression was not significantly different in 1*g* *vs.* MMG-cultured lymphocytes. In order to determine if the differential expression of the calcium-independent isoforms was linked to locomotion inhibition in MMG, they were blocked in 1*g* cultures by specific inhibitors. Considerable optimization was necessary to arrive at doses that were not toxic to cells and yet successfully inhibited the PKC isoforms. It is anticipated that this approach will determine the participation of the isoforms in normal locomotion and set the strategy for elucidating the mechanism by which MMG modulates the expression and possibly the function of PKC in lymphocyte locomotion.

THE ACTIN CYTOSKELETON: A MOLECULAR SIGNATURE FOR GRAVITY SENSITIVITY OF HUMAN BLOOD CELLS

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Experiments over the past two decades clearly demonstrate altered cellular responses in microgravity culture and in ground-based models of microgravity. For example, early space flight experiments indicated that *in vitro* activation of T-lymphocytes results in lack of proliferative response. Our experiments on NASA/ESA Shuttle-Mir Biorack flights demonstrated that this lack of proliferative response is accompanied by dramatic alterations in the response of the tubulin cytoskeleton. More recently we have shown that exposure of the Jurkat T-cell line to as little as 10 seconds of microgravity in parabolic flight results in alteration of the polymerization state of the actin cytoskeleton and its response to activation. The actin cytoskeleton plays a crucial role in signal transduction and activation response of cells, as well as orchestration of mechanical forces inside cells. In the past, however, studies of the actin cytoskeleton in human cells have typically been limited to cell lines and cells purified from human blood samples. Recently we have developed a novel method for measurement of the actin cytoskeleton in whole blood samples without the need for purification of cellular subsets. This new method enables the measurement of the molecular signature of the actin cytoskeleton (the polymerization state of actin and its response to activation) in multiple cellular subsets such as T-lymphocytes, Monocytes, and Neutrophils, using a small quantity of donor blood sample. This measurement of the molecular signature of actin in whole blood samples provides new opportunities for advances in space biology, as well as medical diagnostic of circulating blood cells here on Earth. Preliminary data from hypergravity culture experiments are helping to elucidate the adaptation and response of human cells to hypergravity culture, and they demonstrate that the actin cytoskeleton serves as a molecular signature for gravity sensitivity of human blood cells.

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NOVEL ULTRAHIGH THROUGHPUT FLOW CYTOMETRY FOR CELL AND MOLECULAR ANALYSIS IN UG

GS Spaulding
Spin Diagnostics Inc.

An ultrahigh throughput flow cytometer for cell and molecular analysis was developed. Novel fluidics alleviated the operational dependency on gravity, and increased the throughput by orders of magnitude. Samples are analyzed in a disposable cartridge therein eliminating aerosol hazards and simplifying operations. This analytical tool takes the cost, skill, and complexity out of flow cytometry and enables point-of-care/point-of-use testing.

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ADVANCES IN MICROCULTURING OF CELLS FOR SPACE APPLICATIONS

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We have integrated microfluidic-based technologies that allow the growth, feeding, and monitoring of living cells in 150 microliter culture volumes. The approach allows the potential for smaller culture volumes perhaps by an order of magnitude. This technology, coupled with the extreme sensitivity of modern analytical techniques, allows us to gather certain kinds of data from cells growing in small self-contained systems. These systems are miniature bioreactors that integrate sensors and control systems to regulate feeding and the extraction of wastes. They offer the potential for improved cell growth and more frequent and accurate analysis of cell viability in a hand-held self-contained format.

These miniature automated and self-contained bioreactors implement intermittent feedings and analyze growth curves of cells as a function of environmental stressors such as microgravity, radiation, and electromagnetic environments. This system, called a Bioreporter, is a potential means to understand the living conditions of life in space and its unknown potential threats.

This project attempts to integrate all of the needed components such as microfluidic channels, micropumps, microvalves, mixers, media and reagent supplies, along with optical biochemical sensors into a single micromachined plastic planar housing. These culture systems are designed to be able to support living cells over multiple-week durations and perhaps be designed for partial disposability after use. Prototype systems support bacterial cells in microcultures using a system of microdialysis for feeding and waste extraction. Sensitive optical on-line absorbance and fluorescence measurements of cellular CO2 production and pH are initially used as indicators of metabolic activity. We are experimenting with the integration of sensitive fluorescence and absorbance-based tests into the overall cell culture system. In combination with microfluidic methods of sampling and preparing cells, these basic optical measurement capabilities can be the basis for a potentially large number of molecular analyses on cells and on their secreted products.

Hardware bioreactor designs to date have used multi-layered microfluidic devices manufactured by computer-controlled micro-milling. Present designs integrate pumps, valves, and sensors in a compact plastic package approximately 7 cm x 12 cm x 1 cm thick. These microfluidic miniature bioreactor systems have a variety of potential applications in space as well as terestrial environments including biohazard monitors of air, water, and living environments. They may be a research tool in space because of their small size, light weight, and minimal requirements for user intervention.

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OPTICAL OXYGEN SENSOR FOR USE IN PERFUSION-BASED SYSTEMS: LONG TERM DATA

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The Cell Culture Unit provides an automated, perfusion-based cell culture facility for a wide variety of cell culture studies aboard the International Space Station. In addition to the flight systems, ground units have been developed for gravitational biological study as well as commercial applications in biotechnology and pharmaceutical research. Dissolved pH, CO_2 and O_2 sensing is challenging in a compact, perfusion-based system, particularly when required to perform across a wide range of cell types, from mammalian to yeast. Traditional sensing technologies require frequent recalibration, can be large in size and are often difficult to sterilize. Newly developed optical sensing technologies mitigate these concerns allowing in-situ, long-duration study. An oxygen-sensing optical patch, developed by Polestar Technologies, is attached to a glass surface inside the CCU Cell Specimen Chamber (CSC). A non-invasive LED excitation source and a photo-cell detector reside external to the CSC and measure the sensor patch fluorescence response time which is affected by oxygen concentration. Studies on optical sensors in Saccharomyces cerevisiae yeast cultures, for several subcultures, show rapid responsiveness to fluctuations in oxygen concentration. Additionally, longer-term (60 day) studies in yeast YPD medium at both room temperature and 4°C show sensor stability, with no recalibration. At the time of this writing, tests continue, monitoring oxygen sensor performance for up to 135 days. Similar systems are being developed for pH and CO_2. In summary, automated, timeline-fluorescence, oxygen-sensing tests show promising long-term results. This technology is expected to provide a powerful tool for on-orbit monitoring of cell culture systems.

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CONTINUOUS MONITORING AND CONTROLLING OF GLUCOSE PRESENT IN PERFUSED GTSF-2 MEDIUM IN A ROTATING WALL PERFUSED BIOREACTOR USING AN ON-LINE GLUCOSE SENSOR

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It is well known that glucose is a necessary nutrient for mammalian cell culture. However, mammalian cells have the ability to proliferate under different nutrient environments. Under the conditions normally used in cell cultivation, the cells consume glucose in great excess. Glucose levels can affect cell growth and bioreactor productivity. By controlling glucose at a proper level, cellular metabolism can be altered and can result in reduced glucose consumption as well as in reduced metabolite formation. Therefore, controlling glucose at a proper level becomes important in cell culture. A glucose control system consisting of a glucose sensor, concentrated glucose solution, hardware and software were developed. The system was evaluated in a rotating wall perfused vessel (RWPV) bioreactor (Test Bed Bioreactor), while BHK 21 cells were cultured. The glucose concentration of perfused medium was controlled between 75 and 100 mg/dl for 45 days by an in-line homemade glucose sensor with a computer-controlled system. A glucose stock solution containing high glucose level (4800mg/dl) was used for the glucose injection solution. The in-line glucose sensor is based on a hydrogen peroxide electrode and was continuously and accurately measuring the glucose concentration of GTSF-2 medium in the RWPV bioreactor. Three sets of the two-point calibration were applied to the glucose sensor during the 45 days cell run. The glucose measurement standard error of prediction (SEP) using the sensor are $\pm$ 7 mg/dl for 45 days, as compared to measurement using Beckman Glucose Analyzer.

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OPTICAL MICRO-SENSORS FOR CELLULAR STUDIES OF OXYGEN AND NUTRIENTS

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Microcosm, Inc.

Oxygen and nutrient sensing is fundamental to the understanding of cell growth and metabolism. It is believed that microgravity will affect these processes. A suitable means for studying cell metabolism in space is needed. We will present an optical sensor system that will allow intra- and extracellular oxygen and nutrient measurements. The proposed technology relies on fluorescence lifetime-based sensing techniques and using long-lived fluorophors as sensors. In this method, the source of light is an LED that is modulated by a sinusoidal waveform. Frequency-domain techniques are used to measure the phase shift and the demodulation of the sensor fluorescence that are correlated with the analyte concentration of interest. The performance of select optical sensors for extra- and intracellular measurement of oxygen, pH and glucose will be presented. Design of the sensing system, including a time-resolved inverted miniature microscope for NASA's microgravity biotechnology research, will be discussed.

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ADVANCED BIOLOGICAL TECHNOLOGIES FOR FUNDAMENTAL SPACE BIOLOGY RESEARCH

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A "Technology for Fundamental Space Biology Workshop" was conducted in October, 2002 by NASA Ames Research Center Fundamental Space Biology (FSB) and Astrobionics Programs to envision and brainstorm novel autonomous and miniaturized biological technologies and integrated systems for next-generation space biology research. The workshop focused on technologies that support sampling, imaging, and analysis of a variety of well characterized high use species from cells through small plants and animals. The science drivers for the workshop were derived from the goals and objectives of NASA's Fundamental Space Biology (FSB) flight program. Workshop participants were instructed to consider technologies that could be flown on at least one of the following flight platforms, the ISS, Shuttle, and automated unmanned satellites (free flyers). The workshop addressed the current span of equipment available for FSB research in space and evaluated the potential of making 21st technology available to FSB scientists. Representative fields of interest covered included cell and molecular biology, radiation biology, developmental biology, and organismal biology. Ideally, technologies and integrated systems that support these science disciplines will enable in-situ monitoring and control, autonomous sampling, imaging, analysis, and preservation, and remote modification of protocols as driven by interesting scientific results. As a result of this workshop, several technology development priorities have been identified, and the need to establish a "Technology Integration Agent" function, which includes three elements: Technology Watch, Technology Project Definition, and Technology Research Management. The purpose of this presentation is to present the results of the workshop, describe the technology research priorities initiated since the workshop, introduce the Technology Integration Agent concept, and encourage the evaluation and commentary of the Cell Science community.

GENERATION OF FUNCTIONAL NEUROENDOCRINE TISSUE CONSTRUCTS IN ROTATING WALL VESSEL BIOREACTORS

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Our long-term goal is to engineer functional neuroendocrine tissue constructs, which may become clinically useful as implants in the treatment of debilitating neurodegenerative diseases, such as Parkinson's disease. Using PC12 pheochromocytoma cells as a cellular model, we are utilizing Rotating Wall Vessel (RWV) Bioreactors as a novel cell culture venue for the generation of macroscopic, differentiated 3-D tissue constructs. We are using biochemical, molecular biological and immunological techniques to analyze signal transduction, catecholamine contents, and gene expression in 3-D tissue-like constructs of PC12 cells grown for up to 30 d in RWVs. Vascularization of organoids implanted into s.c. Matrigel plugs in B56 mice was evaluated by fluorescence microscopy. The microgravity-simulating, low-shear culture environment of RWV Bioreactors facilitates the generation of macroscopic, functional neuroendocrine tissue-like assemblies, as assessed by the enhanced production of catecholamines, prolonged activation of specific signaling pathways (erk, p38, jnk) and transcription factors. The constructs engineered in RWV exhibit a unique pattern of gene expression characteristic for the neuroendocrine phenotype.

DIFFERENTIATION OF HUMAN ENDOCHONDRAL CARTILAGE CULTURED IN THE HYDRODYNAMIC FOCUSING BIOREACTOR

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For many years, our laboratory has addressed the question of the effects of gravitational changes on skeletal development. We have shown that changes in gravity, either prenatally, or postnatally, affect the developing skeleton via their effect on development of endochondral cartilage. These studies used tissues received from various shuttle or Biocosmos flights, and from a number of Earth-based systems. For in vivo ground-based studies we have used centrifugation and tail suspension; and for in vitro ones, centrifugation and clinorotation. Our in vitro studies led us to test the NASA developed rotating bioreactors as to their ability to support growth and differentiation of endochondral cartilage. These studies were not concerned with questions of microgravity simulation, but rather with assessing the value of the novel culture system to grow cartilage (human and mouse) to be used in tissue engineering studies. These studies demonstrated that the STLV and HARV bioreactors provide a breakthrough from conventional culture environments in the way the systems support growth and differentiation of chondrocytes. These bioreactors support differentiation of mouse cartilage from undifferentiated limbbud cells and such cartilage is suitable for healing of defects in membranous bone. Human chondrocytes can be cultured as aggregates without the use of substrates, and although during expansion in monolayer culture, human costochondral chondrocytes stop expressing the cartilage phenotype, upon subsequent culture in these bioreactors the cartilage phenotype is expressed. Large cartilaginous nodules with regions of hypertrophy and typical cartilage matrix (as evidenced by metachromasia when stained with Toluidine blue, and immunolocalization of collagen II, aggrecan and cartilage oligomeric protein) are formed. In the present study, a hydrodynamic focusing bioreactor (HFB) was used to culture aggregates of human costochondral chondrocytes for 25 days. The aggregates mostly stayed towards the center of the vessel and fused into one large nodule after 5 days of culture. Rotation was adjusted during culture from 17-30 rpm, an indication of the change in size/mass of the nodule. The large spheroid was fixed, sectioned, and stained with Toluidine blue and antibodies to Collagen II. Cartilage matrix stained positively for collagen II and appeared histologically similar to that seen before, but the metachromatic staining that results from toluidine blue interacting with the glycosamynoglycans (GAGs) of cartilage matrix did not occur in these cultures. Production, secretion and aggregation of GAGs in cartilage are affected by mechanical force, so these results indicate that the chondrocytes in the HFB did not receive sufficient mechanical stimulation, possibly due to their continued state of suspension in the medium, and that the HFB bioreactor may approach closer a spaceflight environment than previously tested bioreactor vessels in terms of load experienced by the cells.

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GROWTH OF HEMATOPOIETIC STEM CELLS IN NASA-RWVs: TOWARDS HEMATOPOIETIC STEM CELL THERAPY FOR EXPLORATION OF SPACE

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Human exploration of deep space depends, in part, on our ability to countermeasure severe/invasive disorders that astronauts experience in space environments. The known symptoms include hematological/cardiac abnormalities, bone and muscle losses, immunodeficiency, neurological disorders, and cancer. Since hematopoietic stem cells (HSCs) differentiate to all types of blood cells, and since the evidence indicates that the HSCs have also a capability to transdifferentiate to various tissues, including muscle, skin, liver, neuronal cells and possibly bone, a hypothesis was advanced in this laboratory that some of the above disorders may be amenable to the hematopoietic stem cell-based therapy, herein called as hematopoietic stem cell therapy (HSCT), so as to maintain astronauts' homeostasis. Thus, the revolutionary aspect of HSCT is to sustain astronauts' homeostasis by the HSCT during deep space exploration, enabling them to "go anywhere at any time" and to come back to earth just as they left. Using mouse models of human anemia (β-thalassemia) as well as spaceflight (hindlimb suspension unloading system), we have obtained feasibility results on HSCT for space anemia, muscle loss, and immunodeficiency. In addition, growth of HSCs in the NASA Rotating Wall Vessel (RWV) culture systems was established, forging the possibility of HSCT in space. Our long-term goal is to automate/robotize the HSCT protocols so that astronauts would be able to treat themselves during long-duration space missions. Such a program would be also beneficial to the earth people as a self-care health system.

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CULTURING DIVERSE HUMAN AND RODENT CELLS IN THE HYDRODYNAMICALLY FOCUSING BIOREACTOR

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We are currently evaluating the feasibility of the Hydrodynamically Focusing Bioreactor (HFB), a novel type of Rotating Wall Vessel (RWV), for the 3-D culture of diverse cells types in suspension. In order to compare the usefulness of the HFB to that of other established RWV types, such as the HARV and STLV, we culture human (microvascular endothelial, ductal breast carcinoma) and rodent (PC12 pheochromocytoma) cells in these three venues for up to 10 days either in suspension or growing on cytodex cell culture beads. In addition to metabolic analyses, we are evaluating the assembly and tissue-specific differentiation of these cells types, using histological, immunohistochemical and molecular biological approaches.

In the initial phase of the evaluation, we discovered several technical shortcomings of a first prototype. After some slight modifications of the original design, most of these problems have been resolved and the system has become much more user-friendly; we will discuss some of the remaining problems and offer solutions.

In terms of its suitability for providing a useful venue for growing three dimensional tissue constructs, the focusing properties of the HFB results in the generation of significantly larger cell aggregates. For example, a 10-day culture of human ductal carcinoma cells, inoculated initially at 106 cells/ml, yielded several dozen spheroids of approximately 2 mm in diameter in both HARV and STLV. By contrast, similar sized aggregates were visible in the HFB within 48 hour. The aggregates then coalesced into a large single aggregate which grow to of > 2 cm within one week.

In our initial assessment we conclude that while the HFB might need some further technological fine-tuning, the addition of hydrodynamic focusing to the established principles of a RWV might lead to significant enhancement of cell assembly and differentiation.

CULTURING PLANT CELLS IN HYDRODYNAMIC FOCUSING BIOREACTOR (HFB)

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Plant cells are important biocatalysts that can be used for production of wide range of secondary metabolites including pharmaceuticals, flavors, fragrances, sweeteners, food colors and pesticides. Bioreactor grown plant cell cultures may prove an excellent source of bio-products because these cultures do not suffer from diseases, pests and climatic restraints, which limit the use of field grown plants. Cell culture conditions in the simulated microgravity environment of Hydrodynamic Focusing Bioreactor (HFB) combine two beneficial factors: low shear stress, which promotes the assembly of tissue-like 3-D constructs; and randomized gravitational vectors, which affect production of medicinal compounds. Close apposition of the plant cells in the absence of shear forces presumably promotes cell-cell contacts, cell aggregation and cell differentiation. This process then might lead to the rapid establishment and expansion of aggregate cultures, which unlike cells cultured in conventional fermentors, are not disrupted by shear forces. Culture conditions in the HFB provide an excellent in vitro system for studying the microenvironmental cues especially intercellular communication on tissue-specific cell assembly, differentiation and function. The main direction of our research is to investigate the effects of microgravity on medicinal plant cell cultures, in particular the events occurring at the membrane level and providing the transduction of primary microgravity effects in the production of medicinal secondary metabolites. In our preliminary investigations, we have noticed photomixotrophic Sandalwood (Santalum album L.) cells cultured in HFB for 7 days can assemble into macroscopic tissue aggregates several millimeters in size, devoid of necrotic cores. By 24 h, cells were forming three-dimensional aggregates. We have monitored fresh weight and dry weight of Sandalwood cell suspensions cultured in HFB. The exponential growth phase appears to begin almost immediately and lasted throughout the seven-day culture period. In addition, the microgravity environment also affected the expression of several proteins, compared to control cell suspensions.

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MICROFLUIDIC DEVICES FOR THE CHEMICAL ANALYSIS OF SINGLE CELLS

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Microfluidic devices for the chemical analysis of single cells Seemingly identical cells are often quite heterogeneous in their chemical composition and in the timing and magnitude of their responses to external stimuli. Determining the chemical composition of individual cells and measuring how that composition changes in response to external stimuli, therefore, are essential to a better understanding of basic cellular functions and intercellular communication. Present methods for examining single cells include flow cytometry and capillary electrophoresis (CE). Flow cytometry, while rapid, is limited to analyzing <4 components in a cell. CE offers powerful separation capabilities, however, it is slow, tedious, and operator intensive. As an alternative to these techniques we are using microfluidic devices. These devices are well suited for meeting the challenges of single cell analysis. They are capable of integrating the various cell handling and processing steps necessary to automate the cell analysis, and separation run times on these devices are significantly shorter while high separation efficiencies are maintained. In addition, injection volumes on the order of the typical mammalian cell volume (1 pL) can be implemented resulting in little analyte dilution, and these devices can be easily coupled with high sensitivity detection schemes such as laser induced fluorescence. We have developed a microfluidic device which integrates cell handling, rapid cell lysis and the electrophoretic separation and detection of the labeled cell contents. The device was demonstrated using Jurkat cells loaded with fluorescent dyes. The loaded cells were hydrodynamically transported from the cell containing reservoir to a region on the microfluidic device where they were rapidly lysed (<33ms) using an electric field. The fluorescent dyes in the cell lysate were automatically injected into a separation channel on the device and detected 3 mm downstream of the injection point in ~2.2 s. The migration time reproducibilities were < 1%. Cell analysis rates of 12 cells/min were demonstrated and are >500 times faster than those reported using CE. This technology is important to advancing NASA's goals of developing micro-analytical systems to provide on-orbit analytical capability for biotechnology investigations in the Biotechnology Facility and to provide support for the human exploration and development of space.

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CELLS GO WITH THE FLOW: CHARACTERIZING THE FLOW AND CHOOSING PERFUSION AND MIXING RATES FOR VARIABLE GRAVITY EXPERIMENTS

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The International Space Station (ISS) Cell Culture Unit (CCU) is designed to support the diverse needs of the cell science community. Growth of cell types including suspension cell cultures (yeast, plant, and mammalian cells), adherent cell cultures, and small tissue specimens are planned in microgravity, on Earth (1- *g*), and on-board the ISS centrifuge from fractional- *g* to 2- *g*. Suspension cells present unique challenges to well controlled cell experiments from microgravity to 2- *g*. To provide uniform culture environment for all the cells and obtain a representative cell or media sample, a homogeneous suspension of cells is required regardless of *g* -level. Homogeneity of the fluid medium is needed for optimal gas and nutrient exchange as well. An environment of low shear stress must also be provided for the cells. These considerations have led to a CCU cell specimen chamber (CSC) that suspends cells with stir bars/paddles while independently controlling nutrient supply and gas exchange with variable perfusion rates within a specialized CSC geometry. To ensure that the flow inside the CSC minimizes stagnant regions and provides a low shear stress environment, three tools have been used to evaluate the flow characteristics: Experimental flow visualization coupled with residence time distribution of fluid dye within the chamber, modeling of the chamber using computational fluid dynamics, and particle imaging velocimetry. Results of these studies have led to improvements in the CSC geometry, including reducing the size of the stir paddle to minimize shear stress and vortices, and configuring the membrane and flow ports to generate a gentle plug flow under conditions of no stirring. Particle imaging velocimetry tests have provided a quantitative comparison between the shear and acceleration forces that cells would experience in the CSC compared to a control erlenmeyer shaker flask. These tools also allow us to define important experiment parameters, including stirring and perfusion rates, for microgravity cell growth as well as 1-*g* and fractional-*g* studies.

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CASSETTE-BASED CELL CULTURE RESEARCH TOOLS FOR MICROGRAVITY EXPERIMENTS

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The automation of cell culture procedures is critical to in vitro cell research on orbiting spacecraft. The ability to place an entire laboratory consisting of incubator, culture vessels, media storage and transfer, feeding and sampling equipment, culture monitoring devices, waste disposal, sample processing capabilities and microscopes, into a few liters of volume is a challenge that must be met without compromising scientific experiment designs and without violating space-flight engineering requirements. A cassette-based system in which these requirements are met has been designed and implemented in preliminary experiments on spacecraft and in laboratory tests. Cassettes are designed to operate under computerized control, and subsystems have undergone continuing refinement. Thermal control is provided by a single-mid-deck-locker equivalent container (“Processing Facility”) that accommodates multiple cassettes. The Processing Facility computer controls all of the activities inside each cassette, and each cassette is treated as a separate experiment. Crew activities consist only of removing and replacing cassettes in the Processing Facility. When telemetry is available, the investigator can read data and perform control functions in each cassette from the ground. When telemetry is not available the Processing Facility computer executes programmed timelines for each cassette. Modularity and versatility of these cell culture tools make it possible to accommodate a wide variety of investigators.

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MOTION OF A POROUS SPHERICAL MICROCARRIER IN A ROTATING WALL VESSEL

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Studies of bone cell function in modeled microgravity employ microcarriers suspended within a rotating bioreactor with cells attached to their outer surface. One limitation of this system is that the number of cells cultured per microcarrier is quite small since the cells are cultured only on the surface and not in the interior. Furthermore, microcarriers often collide with other particles and with the walls of the culture vessel. Importantly, cells on the microcarrier surface form a monolayer that fails to model the three-dimensional characteristics of bone. To overcome the disadvantages inherent in the use of conventional microcarriers, we have designed a new spherical microcarrier in which cells are immobilized in a three dimensional polymer network. In an attempt to quantify the shear stress and acceleration vector experienced by such a porous microcarrier, we have modeled its motion in the RWV (HARV). The essential difference between this and the model for a rigid spherical microcarrier lies with the modification of the effective particle density and the drag coefficient. The effective particle density depends upon the degree of porosity. Also, the drag coefficient here depends on the degree of permeability of the microcarrier. For identical parametric conditions in the HARV, the shear stress at the surface of a porous microcarrier is much smaller compared to the shear stress at the surface of the solid carrier. This is as would be expected. The time taken for particle migration towards the wall of the HARV is higher for a porous particle compared to that for a solid microcarrier. Furthermore, the net acceleration vector experienced by a porous microcarrier is much lesser in magnitude compared to that of a solid carrier. These three observations have implications for simulating microgravity conditions in the laboratory.

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THREE DIMENSIONAL MUSCLE CULTURES AS A MODEL FOR GROUND BASED RESEARCH USING THE SYNTHECON ROTATING CELL CULTURE SYSTEM (RCCS)

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Much of what is known about the mechanisms of cellular function and response to external stimuli has been based on research in two dimensional culture models. We herein describe a three dimensional skeletal muscle model that reproduces in vivo myocyte characteristics with greater fidelity than do two dimensional systems. The C2C12 mouse myoblasts cell line was used to grow bioartificial muscles (BAMs; organoids) according to Vandenburgh with minor modifications. Low passage number cells were trypsinized and resuspended in a collagen, Matrigel mixture. The cell suspension was transferred to a silicon mold with 3mm discs of Velcro anchored by minute pins at both ends. As the cells remodeled the matrix, the BAM detached from the silicon leaving it anchored only by the Velcro anchors. Media was changed to induce differentiation after two days. At four to five days the cells formed contracting structures resembling muscle strips. Mature BAMs were characterized in the following ways and compared with sister cultures grown in two dimensions: 1) cellular dimensions (light microscope and imaging software [SCION]), 2) immunohistochemistry (confocal microscopy) 3) light microscopy (H&E) of longitudinal and cross sections in paraffin, 4) EM (method of Reedy & Reedy, (J. Mol. Bio. 1988)), 5) total RNA, and 6) protein (western blotting). Staining for myosin heavy chain (MHC) showed myotubes aligned along the long axis of the BAM but with staining only visible on the surface. H&E and EM confirmed the absence of cells in the center of the organoid. However, EM examination revealed well-formed sarcomeres with visible Z- and M-lines, A- and I-bands, and H-zones as well as active myoblast fusion zones at the surface. Two-dimensional cultures prepared for EM failed to show the same ordered structures. Experiments with varying collagen concentrations revealed significant variations in the ultrastructure of the organoids. Experiments with various input cell densities revealed that the greater the starting cell number, the smaller the diameter of the resulting BAM (1x106 = 1.7 mm ± 0.11: 8x106 = 0.94 mm ± 0.06), presumably indicating that the greater the starting number of cells, the more remodeling of the matrix occurs. In preliminary experiments, BAMs cultured in the RCCS versus unit gravity controls show lower proliferating cell nuclear antigen (PCNA) and MHC expression. In summary, we have characterized a model that is more similar to functional muscle tissue than previously used monolayer cultures. Future directions include making modifications that will improve their viability in the RCCS and functional characterization studies.

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FLUENT MODELING FOR THE HFB-S

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Efforts are currently in progress to use the commercial CFD package FLUENT to model the motion of gas bubbles in the HFB-S. The model capabilities will be tested with data from recent KC-135 low gravity flights. Measured acceleration data are used in the unsteady non-inertial model to predict bubble motion. Model results are compared with video taped images (using 3 views) from the KC-135 experiments. Results from the flight experiments and some vessel modifications will be discussed.

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FLUID DYNAMIC INVESTIGATIONS OF THE HFB-S

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The Hydro-Focusing Bioreactor for Space (HFB-S) is being developed as an improved system for the cultivation of cell cultures in micro-gravity. In particular, the HFB-S will have improved gas bubble removal capabilities over the RWPV flown on previous Shuttle and MIR missions. Analyses include Oxygen distribution and transport characteristics, cell assemblage motions as they relate to mass transport and mechanical stresses, and gas bubble dynamics.

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ABNORMAL DEVELOPMENTAL EFFECTS FOLLOWING PROLONGED EMBRYONIC EXPOSURE TO A ROTATING CELL CULTURE SYSTEM (RCCS) ENVIRONMENT, IN THE CYPRINIFORM FISH, RIVULUS MARMORATUS

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Embryos of the cypriniform fish, Rivulus marmoratus, were maintained either in a static container or rotating cell culture system (RCCS) to assess the effects of prolonged exposure to RCCS conditions. Experimental embryos were placed in the RCCS shortly after fertilization and maintained until hatching, approximately 21 days later. Survival of viable embryos was 100% in both experimental and control groups. Control post-hatchlings exhibited normal morphology and locomotory movements associated with swimming, as well as successful visual tracking and prey capture of live Artemia nauplia. In addition, they were highly responsive to tactile stimuli. In contrast, experimental fish exhibited extensive curvature of the axial skeleton, abnormal elongation of the lower jaw and marked interorbital cranial depression. Experimental fish were also unresponsive to tactile stimuli and failed to visually track or capture live prey. All experimental fish died within 7 days of hatching. A subsequent histological examination of the brains of both experimental and control individuals revealed a conspicuous lack of neural aggregations within the white matter regions of experimental animals, as well as a reduction in ventricle volume. In addition among experimental fish, retinal layers of the eye were expanded resulting in either the lack of development, or occlusion, of the vitreous humor region, likely rendering image formation impossible.

RAPID EXPRESSION OF NEURONAL MARKERS BY SERTOLI-NEURON-AGGREGATED CELLS (SNAC) TISSUE CONSTRUCTS

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Transplants of fetal dopaminergic neurons into the striatum have been shown to alleviate the symptoms of Parkinson's disease in humans and in animal models of the disease. NT2 cells differentiated for 4-5 wks with retinoic acid into postmitotic immature neurons (NT2N or hNT neurons) have successfully been used to repair stroke and Parkinson's disease induced behavioral deficits. We aggregated these human NT2 cells with rat Sertoli cells to form a tissue construct suitable for transplantation therapy for Parkinson's disease. The Sertoli cell provides growth factors to the NT2 cells and should provide immune privilege to the transplant. Sertoli cells isolated from 15 day-old rats were co-cultured from three to seven days with NT2 cells in a High Aspect Ratio Vessel rotating cell culture system. Sertoli Neuron-Aggregated-Cell (SNAC) constructs were characterized by immunohistochemistry, western blot analysis, or electron microscopy. Sertoli and NT2 cells in SNACs were well organized, with tight junctions between cells on the SNAC periphery. Numerous mitotic figures, in rapidly dividing NT2 cells, were also seen throughout the SNAC. Identification of Sertoli cells with an antibody against Müllerian Inhibitory Substance (MIS) revealed that they are randomly distributed throughout the SNAC. However, the typical arraignment was for postmitotic Sertoli cells to act as a nexus for the growth of NT2 cells. This was verified with double labeling of SNACs with MIS and antibodies against human mitochondria. NT2 cells in SNACs, cultured in DMEM with 10% serum for one week, were immunopositive for type III beta-tubulin, but not for tyrosine hydroxylase (TH) or glutamic acid decarboxylase (GAD). However, at two weeks, NT2 cells within the SNAC were both TH positive and GAD positive. The addition of 1% Matrigel ® to the culture medium resulted in NT2 cells became TH immunopositive within three days of culture. This effect likely was due to the stimulatory effect of Matrigel on Sertoli cells, as no neuronal differentiation of NT2 cells occurred in DMEM medium containing 10% serum 1% Matrigel. Additionally, TUC-4, a protein present in developing neurons, glial fibrillary acidic protein, and NEU-N, a nuclear protein found early in developing neurons, were also present in 3 day old SNACS. These results suggest that aggregation of Sertoli cells with NT2 cells in the HARV may hasten neurilization of NT2 cells.

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MYOBLAST DIFFERENTIATION UNDER FLOW CONDITIONS

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C2C12 murine myoblast differentiation was studied as model system for *in vitro* cultivation of mammalian adherent cells using the Cell Culture Unit hardware. The Cell Culture Unit (CCU) is a perfusion-based cell culture system with programmable flow rates being developed to study the fundamental role of gravity in biological systems aboard the International Space Station. The culture conditions required to support C2C12 attachment, proliferation, differentiation and fusion into myotubes involve efficient transport of nutrients, gases and metabolites in conjunction with minimal hydrodynamic shear. The flow conditions were selected to maintain set levels of oxygen (80-160 mmHg) and pH (7.2-7.4) at an average hydrodynamic shear acting on the cells of < 0.002 Pa (0.02 dynes/cm^2). Cell fusion into multinucleated myotubes and myogenesis strongly depended on medium composition, medium volume, flow regimes, and regimes of medium exchange. Overall, low medium volume per unit cell mass, intermittent flow, periodic and partial medium exchange increased the rate of cell fusion, as well as the density, length and thickness of myotubes formed under flow conditions. Furthermore, cell attachment and proliferation were comparable to static controls but were affected by the attachment surface and medium composition. The ability to change the cell growth surface, flow rate, and medium exchange parameters in the CCU has demonstrated the influence of these parameters on C2C12 differentiation, and will enable the spaceflight investigator to define conditions for their specific cell type.

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INTEGRIN-MEDIATED BONE CELL PROLIFERATIVE RESPONSE TO CONSTANT HYPERGRAVITY

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Bone is a dynamic tissue that changes in strength and density in response to changes in mechanical stimulation. The underlying cellular mechanisms for these responses, however, are still poorly understood. Existing studies of cellular mechanical stimulation by other forces such as flow and substrate deformation suggest that integrin adhesion to the extracellular matrix (ECM) may play a key role in mechanotransduction. Bone cells may also respond to mechanical forces including the constant force of gravity via ECM/integrin-initiated signaling pathways. Using cell culture centrifugation as a source of constant applied force, we found that primary rat osteoblast adhesion to fibronectin at 50-g promotes increased integrin focal adhesion number, focal adhesion kinase phosphorylation, cell survival and proliferation. To determine whether the proliferative response of osteoblasts to centrifugation is mediated by particular integrins, we examined osteoblast growth in various ECMs. The NASA-developed cell culture centrifuge consists of a modified 1-ft-diameter vented Eppendorf 5804 centrifuge inside an environmental cell culture chamber with heating, refrigeration, humidity and CO2 controls. We hypothesized that specific ECMs but not uncoated tissue-culture plastic would stimulate gravity-induced hyper-proliferation. If true, the hyper-proliferation response is likely to be mediated by specific integrins and their signaling complexes. Proliferation of primary osteoblasts grown on fibronectin, laminin, collagen Type I, and collagen Type IV was enhanced when subjected to continuous 24 hour 50-g hypergravity stimulus as compared with the 1-g control. No such increase was observed for cells grown on uncoated surfaces. Furthermore the proliferative response was greatest for cells on collagen Type I (1.4 fold increase over 1g control), suggesting that alpha1beta1, alpha2beta1, alpha3beta1 and alphavbeta1 integrins may be most significantly involved. These results suggest that specific ECM-integrin signaling in hypergravity conditions upregulate cell survival and proliferation pathways. New studies now underway are focused on determining the role of specific integrin signaling pathways in the observed force-induced increase in osteoblast proliferation.

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TRANSGENIC MOUSE MODEL OF ALTERED INTEGRIN SIGNALING IN OSTEOBLASTS: SKELETAL PHENOTYPE AND RESPONSES TO DISUSE

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Prolonged spaceflight, bedrest, and immobilization lead to impaired function of bone forming cells (osteoblasts) and a deficit in bone mass (osteopenia), thereby posing an increased risk of fracture. Abundant evidence obtained from cell culture experiments demonstrates that interactions between extracellular matrix proteins, adhesion proteins (integrins), and associated cytoskeletal and signaling proteins participate in mechanotransduction. To test the relevance of these findings for the intact organism, transgenic mice (TG) were generated by expressing a dominant negative form of integrin under the control of the osteocalcin promoter, thereby targeting expression of the transgene to osteoblasts. To evaluate the skeletal sensitivity of these mice to skeletal disuse, wildtype (WT) and TG female mice were hindlimb unloaded by tail suspension for 4 weeks either during growth (1 mo old) or adulthood (7 mo old). Whereas skeletal mass and strength were similar in WT and TG mice during growth, adult TG mice displayed reduced bone mass and strength compared to WT mice. After 4 weeks of hindlimb unloading, there were no significant differences in body mass or adrenal mass between unloaded and normally loaded, control mice of either age or genotype, suggesting that hindlimb unloading does not cause a sustained stress response. Hindlimb unloading caused changes in bone mass and mineral content both of the forelimbs (which are weightbearing in this model), as well as the hindlimbs (which do not bear weight), in both WT and TG mice. In adults, hindlimb unloading reduced bone strength in both WT and TG mice, although this effect was less severe in TG mice compared to WT mice. In sum, systemic factors may contribute to the skeletal responses to hindlimb unloading of mice, and the skeletal sensitivity to reduced weightbearing is similar in TG and WT mice. Our results indicate that perturbed integrin signaling in osteoblasts appears to cause osteopenia and reduce bone strength in adults, which may influence the skeletal responses to altered patterns of weightbearing.

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ENDOTHELIAL CELL ACTIVATION AND DIFFERENTIATION IN DEFINED 3D HUMAN BIOMATRIX CULTURE MODELS

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Tissue growth and function critically depend upon host extracellular matrix (ECM) and growth factor (GF) signaling events. We created a unique human biomatrix (Amgel*) composed of the major ECM proteins but generally free of GFs and proteases. The defined physiologic milieu of Amgel supports growth and survival of a variety of human cell types. Amgel bioassay systems have allowed the evaluation of phenotypic properties of target cells including adhesion, motility and invasion. In this study, we developed 3D human cell-matrix coculture models that mimic the neovascularization cascade in vitro. These include: Amgel disc-type (1-2 mm) and Amgel microbead (10-20 ul size) systems formulated with or without FGF. Human vascular endothelial cells (EC) were cultured in 3D Amgel systems using a 50-ml rotary bioreactor for 1-10 days. In another design, VEGF-secreting human cells or their media were used for bioassays. Both early and late morphologic events were examined using real-time digital imaging techniques. Results show that disc or bead systems developed with 5 mg/ml of Amgel actively support the growth (MTT assay) of EC without inducing differentiation. Typical 3D Amgel-EC cultures exhibited an endothelium-like monolayer morphology. Moreover, EC activation and differentiation (sprout/capillary formation) occurs only in the presence of specific GFs. Defined cocultures on FGF-formulated Amgel (60 ng/ml) stimulated sprout/ capillary formation in a dose- and time-dependent manner. Control cultures on Type-I collagen gel showed poor EC growth (<60% viability). The initial (days 1-3) mitogenic and late (days 4-10) angiogenic) responses paralleled the sustained release of FGF (or VEGF) from the Amgel biomatrix. The physiologic nature of this 3D functional model should thus allow the dissection of important human cell-cell and cell-matrix interactions. In addition to vascular studies, this new 3D biomatrix system will be valuable to biomedical engineering as well as microgravity-related research. [*patent pending]

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THE USE OF RNAI TO INVESTIGATE GENE FUNCTION DURING POLARITY DEVELOPMENT IN CERATOPTERIS RICHARDII

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We are developing RNA silencing as a technique to investigate gene function during gravity directed polarity development in the single celled spores of *Ceratopteris richardii*. In order to determine if RNA silencing, a post-transcriptional gene silencing mechanism, may be used in this system, we examined whether or not protein translation was required for spore germination. ^{35}S labeled methionine incorporation revealed there was significant protein synthesis during the first 24 h of germination, and transient cycloheximide treatments delayed or prevented spore germination. These results indicate that new protein synthesis is needed for germination progression, and thus, that RNA silencing may be used to examine the role of specific genes during germination in Ceratopteris. We incubated germinating spores with various concentrations of double stranded RNA constructs derived from the sequences of three genes from Ceratopteris: a calmodulin, a calmodulin-domain protein kinase, and a profilin. dsRNA concentrations of 0.05 mg mL^{-1} to 0.10 mg mL^{-1} specifically suppress the expression of the target gene at the mRNA level. Suppression can last up to 120 h, indicating that this technique may be useful for studying the events of gravity perception and response, which occur during a limited period between 6 and 24 h after initiation of germination. These results indicate that RNA silencing is a promising approach for investigating the molecular basis of gravity perception and signal transduction in *Ceratopteris richardii*.

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EARLY T-CELL ACTIVATION MEDIATED BY PKA/MAPK.

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It has been recognized from the early days of spaceflight that microgravity blunts the immune response in humans. Studies by Cogoli have demonstrated that the most likely source of inhibition occurs early in T-cell activation. In preparation for STS-107 experiment, we have conducted ground studies of signal transduction of activation. Since expression of interleukin-2 and its receptor subunits, alpha , beta and gamma are essential for T-cell lymphocyte proliferation; we investigated early induction of these genes. IL-2, IL-2R gamma and IFNgamma were significantly induced at 2h, and IL-2R alpha at 4h after activation; IL-2Rbeta expression was constitutive. The induced gene expression of IL-2, IL-2Rgamma and IFNgamma was significantly inhibited by H-89 (specific PKA inhibitor) to levels equal to or greater than 76% inhibition. PKC inhibition significantly reduced IFNgamma, IL-2 and IL-2Ralpha gene expression by approximately 40%, but did not affect IL-2Rbeta or gamma subunits. In order to further study regulation of IFNgamma, IL-2 and IL-2Ralpha, PMA-stimulated Jurkat cells were analyzed. We found that H-89 inhibited IFNgamma, IL-2 and IL-2Ralpha gene expression, with no effect on 18S. Analysis of proteins showed that H-89 inhibited synthesis of IFNgamma, IL-2 and IL-2Ralpha. MAPK phoshorylation was activated during early T-cell activation moreover, specific MAPK inhibitors blocked activation of the IL-2 R units. In addition, analysis of gene expression in S49 wild-type and S49 kin- T-cells, which are PKA-deficient, show a dependence on PKA for expression of IL-2Ralpha and IFNgamma. H-89 inhibited gene expression of IL-2Ralpha and IFNgamma in S49 wild type cells; IL-2Ralpha was constitutive. In contrast, IFNgamma and IL-2Ralpha were not induced in untreated S49 kin- cells As expected, forskolin could not induce gene expression of IL-2Ralpha or IFNgamma in the mutant cells. These results suggest that T-cell activation, which is dependent on IL-2Ralpha, IL-2 and IFNgamma mRNA expression, is mediated by MAPK via both the PKC and PKA signaling pathways during early stages of activation.

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CELL CYCLE PROPERTIES OF MOUSE L1210 CELLS IN A ROTATING CULTURE VESSEL

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Cell cycle properties of mouse L1210 lymphocytic leukemia cells are being investigated during continuous, steady-state culture in a rotating vessel. The cells growing in the vessel (approximately 5 x 10^7) were attached to a 100-cm^2 porous surface and perfused continuously with culture medium. The culture vessel was constructed such that each division of an attached cell resulted in the release of one newborn daughter cell from the vessel while the other daughter cell remained attached to the surface. As a result, the culture vessel maintained a constant cell number, i.e., enabled long-term continuous culture, while releasing 5 x 10^7 newborn cells each 10-hour generation time. This process continued for many generations, and the released newborn cells grew synchronously through the cell cycle. The cell cycle properties analyzed during rotation include mitotic cycle phase durations, cell sizes, DNA distributions in the cell cycle, gene expression, and DNA segregation between daughter cells. The effects of rotation on the cycle can be clearly identified with this system since the same culture vessel serves as the nonrotating control. The overall findings are that the cells rotating about the horizontal axis grew and progressed through the cell cycle in the same manner as, or faster than, the stationary controls for many generations. Detailed findings on all cell cycle properties will be presented.

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MODELED MICROGRAVITY PROMOTES AGGREGATION AND PROLIFERATION OF NEURAL PRECURSOR CELLS CULTURED IN THREE-DIMENSIONAL COLLAGEN GELS

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While the growth of hematopoietic stem cells and osteoblasts is suppressed substantially by spaceflight or simulated microgravity, the effects of microgravity on neural stem cell development remain relatively unknown. To address this issue, we have proposed a cell-hydrogel-bioreactor system, in which primary neural precursor cells isolated from embryonic day 13 rat cortical neuroepithelium were immobilized in type I collagen gels. The resulting cell-collagen constructs were cultured in a NASA-designed rotating wall vessel (RWV) bioreactor or under static 1g conditions for up to 4 weeks in a serum-free medium containing basic fibroblast growth factor. The collagen-entrapped neural precursor cells cultured under both conditions actively proliferated and differentiated into neurons and astrocytes. The cells cultured in RWV formed larger aggregates than those in static conditions. A 5-bromo-2'deoxyuridine (BrdU) incorporation assay showed a rapid increase in proliferating cells in the first week and a decline in the third week. After dissociating cells from collagen gels with collagenase, cell cycle analysis was carried out using flow cytometry and showed that under RWV conditions there were a higher proliferating population of cells and a lower population in the G1 phase as compared to static conditions. In addition, TUNEL enzymatic labeling, live/dead staining and flow cytometry showed less cell death under RWV conditions. These results suggest that during neurogenesis proliferating neural precursor cells are sensitive to microgravity, which promotes cell-cell interaction and proliferation in a 3D collagen gel. A microgravity environment may be beneficial for growing neural precursor cells in 3D matrices.

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CHARACTERISTICS OF TUMOR AND HOST CELLS IN 3-D ANALOG MICROGRAVITY ENVIRONMENT

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Co-cultures of three-dimensional (3-D) constructs of one cell type with dispersed cells of a second cell type in low-shear rotating suspension cultures in analog microgravity environment have been used to investigate invasive properties of normal and malignant cell types. We have shown that the epithelial and endothelial cells undergo a switch in characteristics when grown in an in vitro 3-D environment, that mimics the in vivo host environment as compared with conventional two-dimensional (2-D) monolayer cultures. Histological preparations and immunohistochemical staining procedures of cocultured harvests demonstrated various markers of interest: like collagen vimentin, mucin, elastin, fibrin, fibrinogen, cytokeratin, adhesion molecules and various angiogenic factors by tumor cells from gynecological cancer patients along with fibroblasts, endothelial cells and patient-derived mononuclear cells (n=8). The production of interleukin-2, interleukin-6, interleukin -8, vascular endothelial cell growth factor, basic fibroblast growth factor, and angiogenin was studied by using ELISA and RT-PCR. Human umbilical vein-derived endothelial cell (HUVEC) were used to study the mitogenic response of the conditioned medium collected from 3-D monocultures and cocultures during proliferation and migration assays. The conditioned medium collected from 3-D cocultures of cancer cells also 1) increased the expression of message levels of vascular endothelial growth factor and its receptor flt-1 and KDR was observed by HUVEC, and 2) increased the expression of intracellular and vascular cell adhesion molecules on the surface of HUVEC, when measured by using Live cell ELISA assays and immunofluorescent staining as compared with 3-D monocultures of normal epithelial cells. There was an increase in production of 1) enzymatic activity that could generate bioactive angiostatin from purified human plasminogen. This coculture system can be used to study the effectiveness of various antiangiogenic agents on endothelial cell proliferation and migration and also the interaction of multiple cell types in a cost effective fashion, since it provides new insights into the invasive process and its effects on both invading and invaded cells.

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SPATIAL ORGANIZATION OF PROSTATE CANCER SPHEROIDS IN A ROTATING-WALL VESSEL AND LIQUID-OVERLAY CULTURE

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We have compared the spatial organization of well (LNCaP) and poorly (DU 145) differentiated prostate cancer spheroids in a mixed culture within a high-aspect rotating wall vessel (HARV) and in a static liquid-overlay culture. LNCaP spheroids were more compact than DU 145 spheroids. LNCaP nuclei, at their centroid, were separated 16-19 micrometer vs. 21-27 micrometer for DU 145 cultures ($p<0.001$). After 14 days of culture, mixing in the HARV produced larger spheroids than the static control but did not significantly affect intraspheroidal spacing. Trends in size and spacing are consistent with the adhesive properties of the two cell lines: LNCaP cells express more E-cadherin, aggregate faster and dissociate slower. Intraspheroidal spacing is a measure of passive diffusion of nutrients and wastes within tissue and, in a mixed culture, is more critical to proliferation than to viability. Using Ki 67 as a biomarker for proliferation, we observed that less compact spheroids were more proliferative: the percentage of Ki 67-positive cells was 51% for DU 145/HARV on average; 37%, DU 145/control; 20%, LNCaP/HARV; and 36%, LNCaP/control. In contrast, mixing in the HARV results in a similar (125%) increase in viable cell thickness for both cell lines due to greater diffusion. The relationship between proliferation and differentiation in this work is counterintuitive. In prostatic epithelium, proliferation occurs more frequently in the basal rather than luminal layer, but we detected the strongest intrinsic staining for markers of the luminal phenotype, cytokeratins 8 and 18, in spheroids with the largest content of proliferative cells, i.e., HARV cultures of DU 145 spheroids. Perhaps, the intercellular communication that is enhanced during differentiation may favor a more aggressive phenotype from the prospective of proliferation. This finding is support by other investigations of metastatic progression.

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DRUG RESPONSIVENESS OF HUMAN OVARIAN TUMOR CELLS CULTURED AS THREE- DIMENSIONAL AGGREGATES

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Spatial orientation affects a variety of parameters critical to cellular growth regulation and responsiveness to intra- and extracellular factors. We have been investigating the influence of three-dimensional growth on the chemotherapeutic responsiveness of ovarian tumor cells. These studies utilize two hormonally responsive human ovarian tumor cells lines, OVCAR-3 and LN-1 cells. Dose response studies revealed that both cell lines when cultured as three-dimensional aggregates in the high aspect rotating wall vessel (HARV) exhibit three-fold or greater resistance to taxol induced killing, as compared to cells grown in two-dimensional monolayer culture. Determination of cell death demonstrated that LN-1 cells appear to undergo both apoptosis and necrosis in response to high dose taxol exposure, while OVCAR-3 cells respond to taxol-induced toxicity predominantly via necrosis. Cytotoxicity induced by taxol was associated altered cell cycle kinetics in conjunction with declining levels of cyclin proteins; levels of CA-125 antigen were relatively unaffected by taxol exposure. Responses induced by taxol were augmented in the presence of mifepristone, suggesting that combination therapy with antihormonal agents administered with taxol could be explored as a plausible treatment option for hormone receptor expressing ovarian tumors.

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ISS CELL SCIENCE RESEARCH: LESSONS LEARNED FROM CBOSS

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CBOSS was a multi-investigator flight experiment on board ISS 7A.1 that included MIP-101 human poorly differentiated colorectal carcinoma cells as well as three other cell types in stationary culture. In two prior Shuttle flights in the Rotating Wall Perfused Vessel MIP-101 cells proliferated and expressed differentiation markers in cultures that were initiated on the ground and taken to space powered. These results and ground experiments suggested the hypothesis that non- or minimally rotated culture decreased apoptosis in these cells. To assess this hypothesis on ISS cell cultures were initiated from frozen stocks and cultured in replicate bag cultures under sterile conditions. Cells were cryopreserved and stored per NASA-approved procedures. The inoculations of the bags and the recovery were performed as per protocol. However, there was essentially no growth in the cultures over the 5 days of cultivation. Extensive post-flight analysis suggests that several factors contributed to the loss of this experiment. A major contributor is that cells cryopreserved in DMSO generate heat when diluted with aqueous tissue culture medium and this heat of fusion (as much as 15°C on earth) is not dissipated in microgravity because gravity-dependent convection is minimal on orbit. In addition, post flight analysis indicated that the samples flown were not optimal for proliferation. Finally, the cell concentration that was minimized to prolong the duration of the experiment may have been too low for this culture. Remedies for the next experiment are 1) to assess different batches of cells closer to flight and select a batch with optimal characteristics for the experiment, 2) to use chilled medium for the initial dilution rather than warm medium to minimize the heat from DMSO dilution, and 3) to use a higher cell concentration for a shorter duration. We will address these concerns in an upcoming flight and provide this experience as important information for cell culture on ISS.

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TUMOR CELLULAR RESPONSE AND GROWTH IN GROUND-BASED MICROGRAVITY ANALOGS AND ROLE OF NUTRITION IN MICROGRAVITY

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Microgravity (MG) induced immune dysfunction in space travel is a major health risk to humans and may endanger future long-term space missions. The incidence of tumor growth and carcinogenesis in microgravity is yet unknown. Commercial and military aviation pilots have increased incidence of cancers and this increase is significantly higher in pilots flying long distance and across the globe. Hence, we investigated the effects of simulated MG (SMG) on tumor growth and tumorigenecity using ground-based in vivo and in vitro models. B16 melanoma cells were cultured in static flask (FL) and rotating wall vessel bioreactors (BIO) to measure growth and properties, melanin production and apoptosis. BIO cultures had 50% decreased growth ($p<0.01$), increased doubling time and a 150% increase in melanin production ($p<0.05$). Flow cytometric analysis showed increased apoptosis in BIO as compared to FL. When BIO cultured melanoma cells were inoculated sc in syngeneic mice there was a significant increase in tumorigenecity as compared to FL cells. Thus SMG may have supported & selected highly tumorigenic cells. It is possible that microgravity, in addition to decreased immune function, may alter tumor cell characteristics and invasiveness. In our previous unit gravity studies, nucleotide supplemented diet significantly decreased tumor growth and metastasis of melanoma in mice. Immunomodulatory nucleotide nutrition has been shown to enhance immune function in mice under SMG environments. Therefore, immunomodulatory nutrition may improve immunosurveillance and prevent carcinogenesis, tumor growth and metastasis in microgravity.

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IDENTIFICATION OF GENES ACTIVATED IN TUMOR-ASSOCIATED STROMA: THREE-DIMENSIONAL CO-CULTURE CELL MODELS RECAPITULATED GENE EXPRESSION PROFILES IN CLINICAL PROSTATE CANCER

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Objectives: After tumor-stroma interaction, that the phenotypes of the interactive cell types can be altered either reversibly (i.e. the altered changes can be maintained transiently) or irreversibly (i.e. the altered changes are permanent). To understand gene expression associated with “stroma reaction” to infiltrating tumor epithelium, we focused our attention on defining prostate or bone stroma reaction to cancer epithelium using 3-D Rotary Wall Vessell (RWV) co-culture models and to assess the relevance of these models to human primary prostate cancer. Methods: cDNA libraries were constructed from laser-capture microdissected primary prostate specimens from normal-, cancer- and benign glandular epithelium-associated stroma. The expression of genes in these libraries serve as gold standards for subsequent cell culture model studies. We evaluated the expression of osteonectin, pro-collagen 1, versican and tenascin in the cDNA libraries by a real-time quantitative PCR and compared these results with the “reactive” stroma obtained from co-culture of either a normal human osteoblast cell line HFOB or a human osteosarcoma cell line MG-63 and an androgen-independent human prostate cancer cell line, C4-2 under 3-D RWV growth condition. Results: We have constructed successfully three high quality stromal cDNA libraries from clinical specimens. Marked upregulation of osteonectin, pro-collagen 1, versican and tenascin was found in cancer- but not benign- or normal-epithelium associated stroma. This upregulation was recapitulated in 3-D co-culture cell models where “reactive” stroma specimens from human osteoblasts were obtained. Conclusions: Gene expression profiles detected in “reactive” stroma obtained from 3-D cell co-culture models recapitulated that observed in clinical specimens. Common genes, such as non-collagenous bone matrix proteins, pro-collagen 1, versican and tenascin may be irreversibly activated in “reactive” prostate stroma in response to infiltrating prostate cancer cells.

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FORCE MEASUREMENTS WITH MOLECULAR TWEEZERS REVEAL REDUCED INTEGRIN-MEDIATED ADHESION IN HUMAN LYMPHOCYTES CULTURED IN SIMULATED MICROGRAVITY

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Rotating Wall Vessel bioreactors were used as an Earth-based system to investigate the impact caused by randomization of gravity on the basic cellular and biochemical mechanisms of integrin-mediated human lymphocyte adhesion. Previously we determined that culture in simulated microgravity for 72 hours caused a defect in cell spreading on integrin alpha4/beta1 substrates but did not alter integrin alpha4/beta1 expression or affinity. We also demonstrated that this effect was not permanent with the cells regaining spreading ability within 48 hours of return to normal culture condition. Using parallel plate flow chambers to measure cellular adhesion over a range of shear forces, we observed a dramatic reduction in cellular avidity in cells cultured in the RWV. The use of molecular tweezers (optical trapping) provides a quantitative method for directly measuring the force required to break molecular interactions. In this method polystyrene beads coated with the alpha4/beta1 substrate fibronectin were captured in a focused laser beam and placed against an immobilized target cell. Laser power was increased until the bead could be separated from the cell and the laser power was then used to determine the molecular force in picoNewtons required to break the fibronectin-alpha4/beta1 interaction. Cells cultured in the RWV showed an average force of separation of 57.5 pN versus 92.2 pN for control cultured cells. These results do not include instances in which there was no measurable interaction between the bead and cell. We found that the number of non-adhesive events was substantially higher (37.8%) in RWV-cultured cells than in controls (9.1%). Therefore, microgravity significantly reduces adhesion between fibronectin and alpha4/beta1 . Future experiments will focus on determining whether this difference is due to anchoring or lateral mobility defects by investigating the association of integrins with other components of the lipid bilayer and cytoskeleton.

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EFFECT OF MODELED MICROGRAVITY ON RAT PANCREATIC ISLETS FOR XENOTRANSPLANTATION

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Type 1 Diabetes remains a major cause of morbidity and mortality despite advances in medical management. Clinical pancreatic islet allotransplantation is a low risk alternative to whole pancreas transplantation and with newer immunosuppression regimens, offers great promise in treating patients with Type 1 diabetes. However, chronic shortages of donor pancreases remain a problem and make xenotransplantation attractive. We developed a mouse islet allograft model and have shown that culture of mouse pancreatic islets in modeled microgravity conditions decreases immunogenicity and maintains islet morphology and function (Transplantation.74: 13-21, 2002). Recently, we have developed an islet xenotransplantation model, in which 500 freshly isolated outbred rat islets or rat islets cultured in stationary dishes or in HARV rotating wall vessel (RWV) bioreactors for 7 days were transplanted under the kidney capsule of C3H streptozotocin-treated diabetic mice. Blood glucose levels and weight are monitored at intervals. Bioreactor-cultured islets had a mean survival time (MST) =20.7 ± 2.63 (SEM) days (n=11, p <0.0001) and dish-cultured islets had a MST=17.8 ± 1.22 days (n=9, p <0.0001), as compared with fresh rat islet xenografts, MST=9.61 ± 0.35 days (n=18). When recipient mice were treated with the immunosuppressive drug FTY720 (2 mg/kg/day x 7 days IP pump), increased graft survival was observed when compared with recipients transplanted with fresh rat islets and FTY720. Specifically, bioreactor-cultured xenografts had a MST=40.33 ± 6.20 days (n=9, p=0.0057) and dish-cultured xenografts had a MST=35.46 ± 3.14 days (n=13, p=0.0300). When compared with untreated recipients, treatment with FTY720 improves xenograft survival in all groups (bioreactor-culture (p=0.0055), dish-culture (p=0.0005), and fresh rat islets (p=0.0001). Previously, we demonstrated that bioreactor-cultured mouse islets develop large channels between islet surface and interior, which may help improve islet morphology and function. Ultrastructural studies are in progress to evaluate impact of bioreactor culture on human islets. Immunohistochemical staining of cultured and fresh mouse and human islets showed greater amounts of insulin, glucagon, and somatostatin in bioreactor-cultured islets than in dish-culture. We conclude that culture improves rat islet:mouse xenograft survival and that treatment of diabetic mouse recipients with FTY720 further improves xenograft survival.

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CIRCADIAN RHYTHM OF HEMATOPOIESIS

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During space flights, astronauts are exposed to abnormal light-dark cycles or reduced light, which lead to circadian dysfunction. In long-duration missions, astronauts further suffer from immunohematological disorders and bone loss. We previously reported that a functional circadian clock exists in bone marrow. This discovery has motivated us to seek to establish the link between the circadian clock and hematopoiesis. The circadian oscillator is composed of interacting feedback-loops formed by both positive- and negative-regulators. In mouse, the negative-regulators include three Period genes (mPer1, mPer2, and mPer3) and two Cryptochrome genes (mCry1 and mCry2). Expression of the negative-regulators is driven by the positive-regulators, such as CLOCK and BMAL1, which form heterodimers via their PAS domains and trans-activate their target genes through binding to the E-box in the 5'-flanking or intronic regions. Our recent discovery of the bone marrow clock provides a plausible molecular basis of circadian rhythms in hematopoiesis. To further elucidate the mechanism, we searched for the hematopoietic transcription factors under the clock control. Here we report the identification of a transcription factor under the direct circadian control in a transient transfection assay using a luciferase reporter gene system. In the assay, CLOCK and BMAL1 dramatically up-regulated the reporter gene expression. The up-regulation depended on the E-boxes in the 5'-flanking region. Deletion or mutation of these E-boxes abolished the CLOCK/BMAL1-depedent transactivation. Furthermore, mPer1, mPer2, and mPer3 each inhibited the CLOCK/BMAL1-depedent transactivation. These results indicate that the bone marrow clock can modulate hematopoiesis through transcription factors expressed in hematopoietic cells and establish a molecular mechanism linking circadian clock with hematopoiesis.

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IMMUNE SYSTEM FUNCTION AND DYSFUNCTION IN THE ROTATING WALL VESSEL AND IN MICROGRAVITY

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We have observed impairments in immune function of lymphoid tissues and cells cultured in the NASA designed RWV, which models some aspects of microgravity. Since impairments of immune function have been documented with space travel, we would like to determine whether the RWV is a useful tool for modeling microgravity effects on the ground. Use of the RWV would allow experiments aimed at understanding immune dysfunction to be conducted with greater ease and frequency. Opportunities to conduct experiments aboard the International Space Station will allow us to determine whether results found in the RWV are duplicated in space. Experiments were modified to accommodate flight conditions and an experiment was conducted on the UF-1 mission, with an identical experiment conducted on the ground. We evaluated proliferation, antibody production, and cytokine production of human lymphocytes activated in microgravity, or activated prior to microgravity. Preliminary results indicate that differences are apparent between flight and ground samples, demonstrating impaired immune responses in flight. Continued analysis will evaluate whether these differences are similar to those seen between RWV and ground samples.

EVALUATION OF GROUND-BASED MIXING IN TEFLON CULTURE BAGS: IMPLICATIONS FOR μ-GRAVITY RESEARCH

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In μ-gravity antibody production, the initiating step in the experiment is to inoculate 14 ml of medium in Teflon culture bags with 1 ml of concentrated cell suspension. Previous trials in μ-gravity demonstrated that the medium and cells were not well mixed based on both the higher antibody levels and the number of cells in an early time point sample. Using solutions of trypan blue, ground-based mixing protocols have been evaluated in order to establish the minimum number of manipulations required to give uniform mixing. A flat bed optical scanner was employed to acquire transmission images of the Teflon culture bag following injection and/or manipulation of the bag. Mixing can be evaluated using a statistical measure, coefficient of variation, of the optical density histogram of the pixels in each image. The resulting coefficient of variation associated with a given mixing procedure was compared with that obtained using a uniformly mixed solution. Mixing on ground can be accomplished with a) 5 withdrawals and re-injections of ~10 ml of medium, b) 5 withdrawals and re-injections of ~ 3 ml of medium followed by 5 seconds of vigorous kneading, or c) 3 withdrawals and re-injections of ~ 3 ml of medium followed by 5 vigorous circular finger massages. Protocol c required only ~ 10 sec to complete. One injection alone, even with manipulation, results in a large volume of unmixed solution at the injection port and may not be suitable for any planned cell culture protocol requiring that a cell inoculum be uniformly distributed in the medium.

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ROTATING WALL VESSEL AS A MODEL FOR INFECTIOUS DISEASE

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GROUND BASED STUDIES OF SACCHAROMYCES CEREVISIAE YEAST GROWTH IN THE CELL CULTURE UNIT

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The Cell Culture Unit (CCU) is a self contained, automated, cell culture system currently being developed to study the fundamental role of gravity in biological systems. It provides a controllable environment (temperature, pH, pO2, nutrient delivery) for both suspension and attachment cell cultures. Achieving a uniform cell suspension and optimal culture conditions (gas and nutrient exchange, gentle hydrodynamic environment) represent unique challenges for the compact sized, perfusion-based CCU cell specimen chambers. *Saccharomyces cerevisiae* (baker's yeast) has been selected to test the CCU capabilities because it is a fast-growing microorganism, well known for its rapid production of carbon dioxide and difficult requirement for gas exchange and cell suspension, and because yeast is a well established model for functional genomics, cell cycle regulation and eukaryotic gene expression. Studies of yeast growth in the CCU were designed both to determine the conditions in the CCU that would provide for culture growth and maintenance, and to characterize hardware performance. Successful culture growth was determined by comparison of CCU cell specimen chamber cultures to control shaker flask cultures. Cultures were assessed for comparable growth rates, cell morphology, and maintenance of cell phenotype. Performance of the hardware was assessed based on its ability to provide adequate gas exchange (by measurement of dissolved oxygen and carbon dioxide levels and the absence of bubble formation), to maintain pH, to provide uniform cell suspension and to prevent cell escape from the cell specimen chamber into the perfusion tubing. Studies routinely maintained the yeast cultures in log growth through four subcultivations. With respect to all criteria, the CCU perfusion-based cultures were comparable to shaker flask controls and demonstrated the ability of the CCU hardware to support yeast cultures for experimentation. Optimization of culture conditions was obtainable due to the control of the perfusion and stirring rates, the direction of stir bar motion, the direction of forward and reverse flow, and control of media re-circulation and fresh medium addition. These studies also demonstrate the feasibility of using highly controlled perfusion systems to support suspension cell cultures for cell biology studies in space.

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MICROARRAY ANALYSIS IDENTIFIES SALMONELLA GENES BELONGING TO THE LOW-SHEAR MODELED MICROGRAVITY REGULON

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The low-shear environment of optimized rotation suspension culture allows both eukaryotic and prokaryotic cells to assume physiologically relevant phenotypes that have led to significant advances in fundamental investigations of medical and biological importance. This culture environment has also been used to model microgravity for ground-based studies regarding the impact of spaceflight on eukaryotic and prokaryotic physiology. We have previously demonstrated that low-shear modeled microgravity (LSMMG) under optimized rotation suspension culture is a novel environmental signal which regulates the virulence, stress resistance, and protein expression levels of Salmonella enterica serovar Typhimurium (Nickerson, et. al. (2000) Infect Immun 68, 3147-52). However, the mechanisms used by the cells of any species, including Salmonella, to sense and respond to LSMMG and identities of the genes involved are unknown. In this study, we used DNA microarrays to elucidate the global transcriptional response of Salmonella to LSMMG. When compared to identical growth conditions under normal gravity (1xg), LSMMG differentially regulated the expression of 163 genes distributed throughout the chromosome, representing functionally diverse groups including transcriptional regulators, virulence factors, LPS biosynthetic enzymes, iron-utilization enzymes and proteins of unknown function. Many of the LSMMG–regulated genes were organized in clusters or operons. The microarray results were further validated by RT-PCR and phenotypic analyses, and they indicate that the ferric uptake regulator (Fur) is involved in the LSMMG response. The results provide important insight about the Salmonella LSMMG response and could provide clues for the functioning of known Salmonella virulence systems, or the identification of uncharacterized bacterial virulence strategies. This represents the first use of whole-genome analysis to identify genes that change expression in response to LSMMG in a prokaryotic organism and the first microarray-based global transcriptional analysis of Salmonella to any environmental signal.

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MOLECULAR BASIS FOR SURVIVAL OF THE HALOPHILE HALOBACTERIUM NRC1 IN SPACE CONDITIONS

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Microorganisms on our planet display tremendous adaptive capacity, some colonizing environments that we usually consider as extreme, such as deep-sea hydrothermal vents and hyper-saline lakes. By studying microbial communities that are adapted to extreme conditions, and establishing the limits to life on our own planet, we may get insights into the potential of other worlds to support life, and thus determine whether life as we know it could exist elsewhere in the galaxy. These ideas are based of the existence of highly desiccation and radiation resistant organisms that could survive the damaging effect of space vacuum and radiation emitted by stars. Halophiles are salt-loving microorganisms growing optimally under extremely high ionic strength, up to ten times the salinity of seawater. They can survive in fluid inclusions within solid salt crystals, and they have been suggested to be the earliest living cells to evolve in nature because of high salt and organic compounds concentration in evaporite environment, giving rise to the primordial soup. In addition, halite and sylvite have been found in chrondrites with a possible origin from asteroidal brines. We have investigated the physiology of survival and recovery of the halophile, *Halobacterium* NRC1, following exposure to desiccation, high vacuum and gamma irradiation, by measuring kinetics of cell survival following treatment. We found that it is extremely resistant to all those treatments. We also found that this halophile is extremely resistant to UV irradiation. We are in the process of obtaining sensitive/resistant mutants to those treatments. Mutant phenotypes under vacuum and radiation treatments will be analyzed in comparison with the wild type. The mutated genes will be identified by genetic complementation studies and genome analysis. In addition to high vacuum, very few studies on the molecular effects of simulated cosmic-ray bombardment have been conducted mainly because of the technical difficulty in reproducing such conditions on earth. Through collaborations with NASA scientists at the Goddard Space Flight Center we have unique access to a Van de Graaff accelerator for simulation of cosmic-ray bombardment. We will investigate the physiology of survival and recovery of *Halobacterium* NRC1 following exposure to proton irradiation. Fourier transform infrared spectroscopy will be used to measure the cells bio-molecular changes following exposure to proton irradiation.

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STS-107 EXPERIMENT BACTER: GROUND EXPERIMENTS

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The growth, physiology and virulence of an Exotoxin A (ETA) producing *P. aeruginosa* ATCC 29260 will be studied in Experiment BACTER on STS-107 A Science Verification Test was done using two sets of four ESA Type I containers and Phorbol experiment cassettes to simulate ground controls, using flight experiment procedures and timelines. In each cassette, 6 culture chambers which contained defined medium were inoculated with a washed bacterial suspension after 9 days storage at 5ºC. Following incubation (37ºC, 24h) culture was terminated and cassettes stored at 5ºC for 7 days before analysis. Total bacterial counts, viable and metabolically active cell numbers, and ETA concentrations were determined on samples from each cassette. Growth from ca. 10^7 (initial) to 10^9 (final) cells/ml was obtained. Similar viable and metabolically active cell numbers were observed. ETA was produced in all cassettes (mean >200 ng/ml) and all cultures hemolysed sheep blood cells. Statistical analysis comparing data for the two sets of cassettes revealed no significant differences, confirming equivalence as expected. The experiment demonstrated that the hardware and culture systems were reliable and ready for flight. Subsequent cultures showed cytotoxicity against a transgenic nematode, *Caenorhabiditis elegans*, with a heat shock inducible lacZ reporter. Samples incubated in a clinostat (80 rpm) to simulate weightlessness indicated variations in protein expression which represent changes in metabolic and physiologic functions or putative pathways for the production of virulence factors. Data from ground experiments in addition to the flight experiment will extend our understanding of the effects of microgravity and spaceflight conditions on bacterial growth, physiology and virulence as they relate to crew health and equipment safety.

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FUNCTIONAL TISSUE ENGINEERING: ROLES OF BIOPHYSICAL FACTORS

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The goal of our research is to engineer functional tissues for *in vitro* research and *in vivo* tissue repair. The effects of biophysical factors on tissue development and function were tested in five studies of engineered musculoskeletal and cardiac tissues, as follows. (1) Composites of engineered cartilage and an osteoconductive support were implanted into large osteochondral defects (7 x 5 x 5 mm) in adult rabbits. Over a period of six months *in vivo*, constructs remodeled such that cartilaginous repair tissues exhibited characteristic structural features (thickness, cell alignment), differentiation markers (collagen type II and glycosaminoglycans [GAG]), and mechanical behavior (Young's moduli). (2) Engineered cartilage (bovine calf chondrocytes/polyglycolic acid [PGA] mesh) was cultured in different mechanical environments (petri dishes, spinner flasks, rotating vessels) in medium supplemented or not with insulin-like growth factor I [IGF-I]). Over one month *in vitro*, mechanical and biochemical factors independently modulated construct properties and in combination yielded tissue better than that obtained by modifying these factors individually. (3) A perfused bioreactor was developed to culture engineered cartilage (bovine calf chondrocytes/PGAmesh) in the presence of physiologic loading (cyclic compression, 5% strain amplitude, 0.3 Hz). Over three months *in vitro,* construct remodeling included the formation of ~1 mm thick, fibrous capsules at the free surfaces and decreases in GAG content and equilibrium moduli that were correlated with longer culture duration and the presence of hydrodynamic shear. (4) A perfused bioreactor was developed to culture engineered ligament (human bone marrow stromal cells/collagen gel or silk bundles) in the presence of physiologic loading (cyclic tension and torsion, intermittently applied). Over one month *in vitro*, constructs cultured with mechanical stimulation exhibited cell alignment in the direction of resultant force, and higher cell densities and higher expression levels of mRNA for tenascin and collagen types I and II than unstimulated controls. (5) A Bio-Stretch apparatus was used to apply cyclic stretch to engineered cardiac tissue (neonatal rat heart cells/derivatized hyaluronic acid knit) in the presence of physiologic loading (cyclic tension, 3 % strain amplitude, ~1.3 Hz). Over one week *in vitro*, constructs cultured with mechanical stimulation exhibited higher DNA contents and higher tensile stiffnesses than unstimulated controls. Together, the above findings imply that biophysical factors can be utilized to modulate the structural and functional properties of engineered tissues. Related studies from the same NASA grant, entitled Microgravity Tissue Engineering, are described in an abstract by G. Vunjak-Novakovic.

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BIOMIMETIC APPROACH TO THE TISSUE ENGINEERING OF FUNCTIONAL MYOCARDIUM

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We hypothesize that functional, clinically sized cardiac constructs containing high and spatially uniform cell densities can be engineered by mimicking in vitro the oxygen transport and electrical stimulation normally present in vivo. Neonatal rat cardiomyocytes were seeded on collagen scaffolds and cultured in perfusion bioreactors that provided interstitial flow of medium through the construct; constructs seeded and cultured in orbitally mixed dishes served as controls. The use of Matrigel® as a vehicle for rapid delivery of cells onto 10 mm diameter x 3 mm thick scaffolds consistently resulted in high cell seeding yields of 80 to 90%. The use of perfusion with alternating medium flow (0.5 to 1.5ml/min) dramatically improved cell viability and spatial uniformity as compared to seeding and cultivation in mixed dishes. Constructs cultured for 1-week in perfusion with unidirectional medium flow (0.5 ml/min) were 1.0 to 1.5 mm thick, consisted of uniformly distributed cells, contained sarcomeric a-actin, troponin I and tropomyosin, and exhibited aerobic cell metabolism (a molar ratio of lactate produced to glucose consumed of 1.0). In contrast, constructs cultured for 1 week in mixed dishes had a peripheral region ~100 μm thick consisting of functionally connected cells and an acellular interior. Cell cycle analysis demonstrated similarities between constructs cultured in perfusion and freshly isolated cardiomyocytes (more cells in S phase than in G2/M phases), whereas constructs cultured in dishes appeared unable to complete the cell cycle (cells accumulated in G2/M phase). Constructs were electrophysiologically functional and contracted synchronously in response to electrical stimulation. Constructs cultivated in perfusion exhibited lower excitation thresholds than constructs cultured in mixed dishes (~3.3 and ~4.5 Volts, respectively). These findings can be explained by differences in oxygen supply, i.e. constructs seeded and cultured in perfused systems are supplied with oxygen by a combination of convection and diffusion across short distances, whereas constructs cultured in conventional systems are supplied with oxygen by diffusion across large distances. We conclude that improved oxygen supply is essential for the cultivation of functional engineered cardiac constructs. Related studies from the same NASA grant, entitled Microgravity Tissue Engineering, are described in abstracts by P. Zandstra and L.E. Freed.

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CHARACTERIZATION OF NOVEL RECOMBINANT COLLAGEN-LIKE PROTEINS

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Fibrillar collagens are attractive materials for production of matrices needed for tissue engineering and drug delivery. It has been previously established that fibrillar collagens consist of domains that are responsible for such processes as interaction with cells, binding of growth factors, and interaction with a number of structural proteins present in the extracellular matrix. In the presented study, we explore the possibility of engineering recombinant collagen-like proteins consisting of a multiplied domain that corresponds to a specific region of the collagen monomer; if “D1,D2,D3,D4” describes the domains of normal collagen, the sequence of “D1,D1,D1,D1” describes one of the recombinant multi-domain collagen-like proteins. Because these “custom-tailored” proteins will have a higher density of sites available for specific interactions, they will have superior properties critical for successful tissue engineering. Due to the unique characteristics of native collagen needed for correct structure and function of this protein, expressing the engineered triple helical collagen-like proteins that consist of multiplied homogenous domains but lack other domains presents a significant challenge. Using a DNA cassette system, we gene engineered a construct that encodes collagen-like protein consisting of multiple D4 regions of human collagen II which, as previously demonstrated, is critical for integrin-mediated interaction between chondrocytes and collagen II. The DNA construct was expressed in HT-1080 cells, and the recombinant multi-D4 collagen was analyzed. The structural integrity of the multi-D4 collagen was analyzed by limited protease digestion. Results demonstrate that the thermal stability of the protein was 41°C, a value similar to the thermal stability of native collagen II. Moreover, the protein served as a substrate for the procollagen C-proteinase/BMP-1 and procollagen N-proteinase, a further indication that the multi-D4 collagen folded into the correct collagen-like structure. Glycosylation of the novel collagen-like protein and the morphology of single monomers was also analyzed. Overall, results demonstrated the feasibility of rational designing of stable and correctly folded recombinant collagen-like proteins suitable for preparation of smart biomaterials.

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DEVELOPMENT OF A MODIFIED PERFUSED BIOREACTOR FOR ANALYSIS OF HORMONE SECRETION IN ISLET CELL CULTURES

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We have developed a modified perfusion-capable model of the rotating bioreactor. The dimensions of the rotating culture chamber were developed by experiments in which clonal islet cell aggregates grown in the chamber were challenged with a stimulus of high glucose, and samples collected sequentially over a time course were analyzed for insulin content. This data was used to assess the optimal volume for the chamber, taking into account the peak onset of insulin secretion in response to the glucose challenge; dead space of the container and time-frame for liquid diffusion in the chamber. The new bioreactor that has been fabricated is a 10 ml chamber and contains a replaceable flat filter that allows for variability in membrane pore size based on desired flow rate. This configuration differs from the older version of the bioreactor incorporating a cylindrical core through the center of the vessel. The new design allows higher flow rate and minimizes clogging of the filter by adherent cells. The perfusion prototype has been manufactured with the following functionality: the entire bench-top manifold is an enclosed chamber that is temperature controlled (37*C) and gassed by carbon dioxide. Sensors continuously monitor temperature and carbon dioxide levels. An automated system permits continuous flow of desired media (growth and test solutions) placed in the manifold across the cell culture in the rotating bioreactor. The effluent is then collected in a time-dependent fashion through a fraction collector for analysis of secreted productions. Alternately, the output can be discarded or recirculated into the culture chamber, for long-term culture protocols – future modifications may allow programmable microprocessor-controlled for automated feeding of cell cultures.

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LIVER TISSUE ENGINEERING IN MICROGRAVITY ENVIRONMENT

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Background: Liver diseases affect the lives of millions. The only available treatment for subjects with end-stage liver disease is liver transplantation. However, due to the shortage of donor livers, there is an urgent need to develop alternative approaches, including liver assist devices, hepatocyte transplantation and xenotranstpantation. While these approaches require a readily available pool of primary hepatocytes, previous efforts have failed to establish long-term cultures of viable and differentiated hepatocytes. *Methods and Results*: Using a NASA-developed bioreactor that creates the unique environment of low shear force and enables 3-D cell growth, we demonstrated that a simulated microgravity environment is conducive for maintaining long term cultures of functional hepatocytes. We observed the formation of liver tissue-like structures (up to 3.0 cm in length) from isolated by collagenase perfusion liver cells. Albumin mRNA was expressed throughout the 60-day culture and co-culture of isolated hepatocytes with endothelial cells stimulate albumin expression. Microsomal functions were demonstrated by activity of the p450 system. To optimize oxygenation and nutritional uptake within growing cellular aggregates the cells were cultured in the presence of biodegradable scaffolds that resulted in improvement of liver remodeling. Tissue-like structures comprised of hepatocytes, biliary epithelial cells and/or progenitor liver cells that were arranged as bile duct-like structures along nascent vascular sprouts and scaffolds. Electron microscopy revealed groups of cohesive hepatocytes surrounded by complex stromal structures and reticulin fibers, bile canaliculi with multiple microvilli, and tight cellular junctions. Hepatocytes cultured in simulated microgravity were examined for markers of ER-stress and apoptosis. We observed decrease in ER stress following reduction of calcium efflux, inhibition of Bip/GRP78 and caspase-12 activation. Subsequent studies demonstrated inhibited activation of effector caspases and apoptosis. Using DNA microarray technology we demonstrated that simulated microgravity modify the expression of several genes (over expression of 85 genes and down regulation of 10 genes) and that microarray technology may provide new understanding of the fundamental biological questions of how gravity affects the development and function of individual cells. *Conclusion*: Simulated microgravity environment is conducive for maintaining long-term cultures of functional hepatocytes. This model system will assist in developing improved protocols for autologous hepatocyte transplantation, gene therapy, liver assist devices, and facilitate studies of liver regeneration and cell-to-cell interactions that occur in vivo.

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GENE EXPRESSION PROFILING OF HUMAN RENAL CORTICAL EPITHELIAL CELLS AT THREE STAGES OF TISSUE ENGINEERING IN THE HYDRODYNAMIC FOCUSING BIOREACTOR (HFB)

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NASA-designed bioreactor systems are being used both in Space and on Earth to advance our understanding of the gravity effects on cells and tissues. Numerous three-dimensional models have been developed from tissue engineering studies in these bioreactors. A seminal report (Hammond et. al.,Physiol. Genomics 3:163-173) on the effect of gravity on gene expression in Human Renal Cortical Epithelial Cells grown as three-dimensional aggregates in a bioreactor in Space indicated 1,632 genes of the 10,000 genes analyzed were regulated. In the present study we used a Hydrodynamic Focusing Bioreactor (HFB) that models microgravity and provides an optimal environment for three-dimensional tissue engineering to assess gene expression at several specific tissue engineering stages. Human Renal Cortical Epithelial Cells were cultured in the HFB for six days on Cytodex-3 beads in the HFB and in a stationary teflon bag control. Cells were harvested from the bioreactors and the bag controls at 24, 72 and 144 hours after inoculation for gene array analysis. These time points correspond to three phases of the tissue culture – attachment, aggregation, and growth - as observed in previous studies. Samples were taken periodically over the six-day period and characterized by light, scanning and transmission electron microscopy for cell attachment, growth, and viability. Results show complete attachment of cells to the beads by 48 hours, and the presence of large cell aggregates by 72 hours in the HFB vessel. A profile of genes was observed to regulate in accord with the major activities of initial cell attachment and aggregation, and with the subsequent growth and differentiation stages.

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